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STUDIES IN MAN ON TESTICULAR AND ADRENAL RESPONSE TO ADRENOCORTICOTROPIN AND HUMAN CHORIONIC GONADOTROPIN*

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ABSTRACT

The present study compares, in the same 17 subjects with normal adrenocortical and testicular function, the response of the urinary total 17-hydroxycorticosteroids and 17-ketosteroids to the slow intravenous administration of adrenocorticotropin (ACTH) and human chorionic gonadotropin (HCG). In addition, 6 patients with Addison's disease, bilateral orchiectomy, bilateral orchiectomy and adrenalectomy, and primary and secondary hypogonadism were studied in order to evaluate: 1) the effect of HCG on adrenocortical secretory activity, 2) the relative contributions to urinary steroid output made by the adrenal cortices and testes, 3) the possible usefulness of changes in urinary 17-ketosteroid excretion as a rapid test of testicular interstitial-cell function, 4) the dose-response effect of intravenously administered HCG, 5) the effect of estrogens and androgens on the excretion of 17-hydroxycorticosteroids and 17-ketosteroids, and 6) the possible synergistic effect of ACTH and HCG on the excretion of these steroids.

The results indicate that intravenous administration of HCG, even in large doses, does not lead to increased urinary excretion of total 17-hydroxycorticosteroids. In addition, there is an obvious difference between the patterns of

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increased urinary 17-ketosteroid excretion following administration of ACTH as compared with HCG. This difference suggests that HCG does not stimulate the adrenal cortex in a manner similar to ACTH. The rapid and significant increase in 17-ketosteroid excretion in normal subjects and the slight increase in the patient with panhypopituitarism are in contrast to the lack of increase in patients with primary hypogonadism. This points to the possible usefulness of this procedure in distinguishing primary from secondary hypogonadism, and in studying testicular interstitial-cell function. Administration of estrogens or androgens does not modify the urinary corticoid levels, and there is no apparent synergism between HCG and ACTH.

INTRODUCTION

HORMONAL interrelationships continue to be a problem of major interest. Earlier studies in man have shown, on the bases of changes in testicular histology and increases in urinary 17-ketosteroid levels, that human chorionic gonadotropin (HCG) stimulates hormonal secretory activity of the testes (1). Insofar as one can differentiate the various activities of the gonadotropins, this material obtained from human pregnancy urine has biologic activity resembling that of luteinizing or interstitial-cell stimulating hormone (1).

There is considerable experimental evidence both for and against a stimulatory effect of human chorionic gonadotropin on adrenocortical secretory activity (2–23). Although impressive evidence has been accumulated to support such a tropic effect, much of it is indirect and speculative (2–10). Direct experimental data, for the most part, support the concept that these gonadotropins do not stimulate the adrenal cortex (11–23). In many of the earlier studies in man, a change in the level of urinary 17-ketosteroid excretion was the sole index of adrenocortical activation; and the capacity of the adrenal cortex to respond to stimulation by adrenocorticotropin (ACTH) was not evaluated.

It appeared worth while to re-evaluate the adrenocortical response to HCG, employing technics which measure more directly adrenocortical secretory activity, and using subjects in whom the functional capacity of the adrenal cortex had been determined by specific stimulation with ACTH. Therefore, the present study was designed to compare, in the same subjects, the response of the urinary total 17-hydroxycorticosteroid and 17-ketosteroid levels to the slow intravenous administration of ACTH and HCG. With this technic, HCG was infused into subjects with normal adrenal cortices and testes in order to assay its effect on adrenocortical secretory activity and to evaluate the possible usefulness of changes in urinary 17-ketosteroid excretion as a relatively rapid test of testicular interstitial-cell function.

These studies have been supplemented by others designed to deter-

mine: 1) the relative contributions to the urinary steroid levels made by the adrenal cortices and testes; 2) the effects of estrogens and androgens on the excretion of 17-hydroxycorticosteroids and 17-ketosteroids; 3) the possible contamination of human chorionic gonadotropin with ACTH; 4) the possibility of nonspecific protein, renal or circulatory effects in causing changes in steroid excretion; 5) the effect of varying doses of intravenously administered human chorionic gonadotropin on steroid excretion; and 6) the possibility of the synergistic or antagonistic action of combined administration of ACTH and HCG on the excretion of steroids.

MATERIALS AND METHODS

Twenty-three men were studied under similar conditions of dietary control and activity on the Metabolic Service of the Birmingham Veterans Administration Hospital. They were classified as follows: *a*) 17 patients without adrenocortical[1] or testicular[2] dysfunction, ranging in age from 22 to 58 years; in 14 of these the diagnosis was rheumatoid arthritis or spondylitis, in 2 it was hyperthyroidism, and in 1 sarcoidosis; *b*) 1 patient, aged 61, with proved Addison's disease and normal testicular function; *c*) 2 patients 49 and 63 years of age, with normal adrenocortical function, who had had an orchiectomy for carcinoma of the prostate; *d*) 1 patient, aged 61, who had undergone bilateral total adrenalectomy and orchiectomy for carcinoma of the prostate; *e*) 1 patient, aged 65, with combined primary testicular atrophy (proved by testicular biopsy and elevation of the urinary gonadotropin level to 4,347 mouse units per twenty-four hours) resulting from mumps, and primary hypothyroidism (proved by unresponsiveness to administration of thyroid-stimulating hormone); and *f*) 1 patient, aged 58, with panhypopituitarism resulting from a chromophobe adenoma.

The intravenous ACTH test was performed one or more times on all but 1 of the patients—the exception being the man in group *a* with sarcoidosis. The test procedure used was a modification of the method of Jenkins and co-workers (24), in which the 24-hour excretions of urinary 17-hydroxycorticosteroids and 17-ketosteroids were determined on one or more control days, two consecutive test days, and one to four days thereafter. On each of the two test days, a dose of 20 to 25 U.S.P. units of lyophilized ACTH dissolved in 1,000 ml. of 5 per cent dextrose and water was administered intravenously over an eight-hour period. Results of 3 of the ACTH tests were not included in the table because of technical difficulties, although the response was considered adequate.

The intravenous HCG test was performed by administering human chorionic gonadotropin ("APL")[3] intravenously over an eight-hour period on two consecutive days in doses of 1,000, 5,000, 10,000, or 20,000 international units dissolved in 1,000 ml. of 5 per cent dextrose and water. Of the patients with normal adrenal cortices and testes, 2 re-

[1] Normal response to administration of ACTH.

[2] Normal libido, secondary sex characteristics, and testicular size.

[3] We are indebted to Doctors John Jewel and J. S. Scanlan, Medical Department, Ayerst Laboratories, for the generous supply of "APL" used in these studies. The material was supplied in 2 forms: 1) Sterile solution (1,000 I.U. per ml.) in 10-cc. vials, and 2) a "Secule" containing 20,000 I.U. Most of the studies were performed with one lot of the sterile solution.

ceived 5,000 units, 1 received 1,000 units, 2 received both 1,000 and 5,000 units, 5 received 10,000 units, 5 received 20,000 units, and 2 received both 10,000 and 20,000 units. The 24-hour excretions of urinary creatinine, 17-hydroxycorticosteroids and 17-ketosteroids were determined on two or more control days, two consecutive test days, and for three to six days thereafter.

All intravenous infusions were started about 8 A.M. All urine samples were collected from 7 A.M. to 7 A.M. and kept refrigerated without preservative.

The 17-ketosteroids were extracted by a modification of the method of Drekter *et al.* (25) and the colorimetry was performed by a modification of the method of Holtorff and Koch (26). Using this method in our laboratory, values have ranged from 8 to 25 mg. per twenty-four hours (with a mean of 15 mg.) for normal male subjects. The 17-hydroxycorticosteroids were determined by a modification of the method of Reddy (27). With this method, values in our laboratory have ranged from 1 to 11 mg. per twenty-four hours (with a mean of 5 mg.) for normal male subjects. The reported results for both urinary steroids are averages of duplicate determinations and are expressed as milligrams per twenty-four hours. The creatinine content of each urine specimen was determined in an attempt to check the accuracy of the collection, and was measured by a modification of the method of Lambert (28). To minimize the effect of minor inaccuracies in the timing of urine collections, the values for each subject were corrected according to the average creatinine content for all their specimens. In every case, however, the corrections were small, and when the creatinine content was obviously so low as to indicate a grossly faulty collection, the values were discarded.

The HCG material used in this study was assayed for ACTH activity by a modification of the method of Sayers *et al.* (29), in which ascorbic-acid depletion in the adrenals of hypophysectomized rats is used as an index of ACTH activity.[4] No ACTH activity could be detected in this HCG, in contrast to other such materials (30). HCG, which had been deactivated by heating at 70° C. for twelve hours, failed to elicit a response in a normal patient who had previously been shown to be responsive to 20,000 units of infused HCG. This procedure has been reported to destroy gonadotropic activity without affecting ACTH activity (30). When the HCG material *in solution* was used, no systemic effects were noted from a single intravenous test in the majority of patients with normal adrenal cortices, nor was there any hypersensitivity reaction following two to four tests on the same subject (31). Two patients with untreated panhypopituitarism had febrile reactions following the intravenous administration of this substance and had to be excluded from the study. Another patient with panhypopituitarism being treated with cortisone had no adverse reaction to the test. One patient with Addison's disease also had a febrile reaction when cortisone was withdrawn, but had no such reaction upon repeating the test after institution of maintenance cortisone therapy. Therefore it would appear that this HCG preparation is usually safe to use intravenously except in patients with untreated adrenocortical insufficiency (whether primary or secondary), in whom one would expect reactions from many nonspecific infusions, such as glucose.[5] In the few instances in which the *dried* HCG material was used, febrile and skin hypersensitivity reactions were frequently observed in all types of patients.

[4] We are indebted to Dr. Robert S. Teague, Professor and Chairman, Department of Pharmacology, the Medical College of Alabama, for the ACTH assays.

[5] Following completion of this study, a patient with congenital adrenocortical hyperplasia resulting in female pseudohermaphrodism had severe chills and fever following the intravenous administration of HCG—but not following intramuscular administration. Again, the febrile reaction might be related to inadequate production of 17-hydroxycorticosteroids.

RESULTS

Subjects with Normal Adrenal Cortices and Testes

ACTH tests (Table 1 and Fig. 1)

Twelve subjects in this category were studied. All responded with an increase in 17-hydroxycorticosteroid excretion on both the first and second days of ACTH administration. The mean increase above the control level was 12 mg. per twenty-four hours on the first day and 17 mg. on the second day, with a return to the control value on the first day after discontinuing ACTH. In 10 of the 12 patients there was an increase in urinary 17-ketosteroids on either the first or the second day. The mean increase was 3 mg. per twenty-four hours on the first day and 4 mg. on the second day of ACTH administration. On the first day after discontinuing ACTH the 17-ketosteroids returned to the control value, and on the second day fell slightly below this level. Based on the results of these tests, all of these patients were judged to have normal adrenocortical function (24).

HCG tests (Tables 2 and 3; Figs. 2 and 3)

All patients in the following groups, except the one with sarcoidosis who was not given ACTH, had normal responses to the ACTH test.

10,000 units (Table 2, Fig. 2). Seven subjects were given 10,000 units of HCG intravenously on two successive days. There was no statistically sig-

TABLE 1. RESPONSE OF URINARY 17-HYDROXYCORTICOSTEROIDS AND 17-KETOSTEROIDS TO INTRAVENOUS ACTH IN 12 SUBJECTS WITH NORMAL ADRENOCORTICAL AND TESTICULAR FUNCTION

(mg. per 24 hrs.)

Patient No.	Age (yrs.)	Control		Intravenous ACTH				After ACTH					
				Day 1		Day 2		1st day		2nd day		3rd day	
		OHCS	KS	OHCS	KS	OHCS	KS	OHCS	KS	OHCS	KS	OHCS	KS
1	24	7.28	24.24	26.84	24.31	34.44	28.17	8.16	13.40				
2	22	6.80	19.20	17.48	17.57	13.18	17.68	3.28	15.30	5.00	11.56	5.37	9.70
3	48	5.43	21.64	20.92	20.32	26.05	32.42	7.50	16.20	4.42	10.11	1.96	10.37
4	47	5.40	9.90	9.58	20.58	9.31	21.09	5.97	18.62	9.77	10.00		
5	39	6.21	13.21	20.62	16.83	31.31	15.21	6.52	14.35	5.99	12.79	5.76	11.30
6	38	5.16	14.78	11.15	16.83	18.80	13.14	7.81	12.91	4.64	11.59	10.21	11.21
8	34	6.47	23.24	31.01	44.67	29.42	46.67	8.50	28.19				
10	45	5.25	13.80	14.65	15.80	29.00	18.44	3.56	13.50	3.53	13.62	8.97	13.20
11	42	6.34	19.95	22.55	29.14	22.90	18.70	11.66	18.72	8.76	11.26	5.90	12.90
13	49	2.46	34.93	8.72	29.75	19.23	27.29	12.31	29.15	2.95	28.38	3.93	32.95
14	28	2.92	17.97	6.80	22.09	11.61	20.15	2.90	19.12	3.30	15.35		
15	58	3.90	10.88	10.88	17.00	20.64	17.01	1.52	17.53	4.07	14.13	6.06	9.93
MEAN		5.30	18.64	16.76	22.03	22.15	22.99	6.64	18.08	5.24*	12.8*	6.02†	13.9†
S.E.		0.4	2.0	2.2	2.4	2.3	2.7	1.0	1.6	0.7	1.7	0.9	2.5

* Ten patients.
† Eight patients.

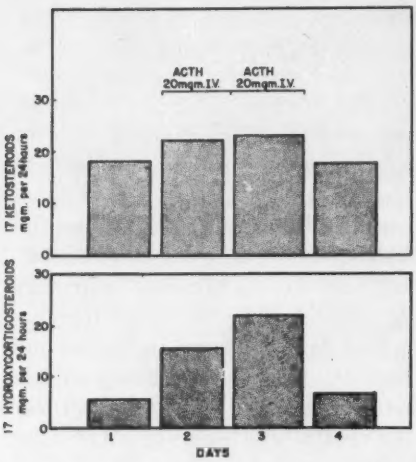

FIG. 1. The mean urinary steroid response to intravenously administered ACTH in 12 male subjects without known adrenocortical or testicular abnormality.

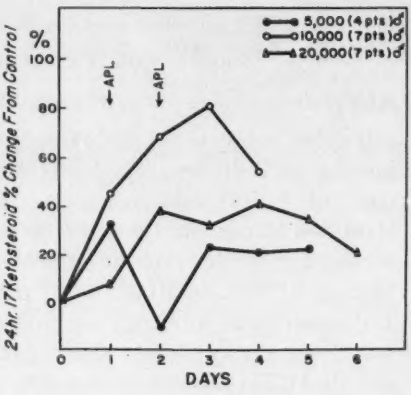

FIG. 2. The mean urinary 17-ketosteroid response to HCG plotted as the percentile change from the control levels following the intravenous administration of 5,000, 10,000 and 20,000 units of HCG to subjects with normal adrenocortical and testicular function.

nificant increase in the mean level of urinary 17-hydroxycorticosteroids, although in 5 of the 7 subjects there was a rise on the first day, and in 2 of the 7 there was a rise on both the first and second days of the test. In *all* patients there was a rise in urinary 17-ketosteroids on either the first or the second day. The mean rise per twenty-four hours above the control level was 5 mg. on the first day and 7 mg. on the second day of HCG administration. On the day following administration there was a continued rise to 9 mg. per twenty-four hours and excretion had not returned to the control level by the second day after the infusion. The rise in 17-ketosteroids was significant at the 5 per cent level during the first and second day of HCG administration.

20,000 units (Table 2, Fig. 2). Seven subjects were tested with 20,000 units of HCG. In 5 there was a rise in urinary 17-hydroxycorticosteroids on the first day, and in 4 of these 5 there was a rise on both the first and second day. The mean rise above the control 24-hour level for the entire group was 1 mg. on the first day and 2 mg. on the second day of HCG administration. These increases were not statistically significant. In 5 of the 7 patients there was a rise in 17-ketosteroids on the first day, and in all 7 there was a rise by the second day of the infusion. The mean increase per twenty-four hours was 1 mg. on the first day, and 5 mg. on the second day. This increase in the mean level was not significant on the first day but was

TABLE 2. URINARY 17-HYDROXYCORTICOSTEROID AND 17-KETOSTEROID RESPONSE TO INTRAVENOUS HCG (10,000 AND 20,000 UNITS) IN SUBJECTS WITH NORMAL ADRENOCORTICAL AND TESTICULAR FUNCTION

(mg. per 24 hrs.)

Patient No.	Age (yrs.)	Dose of HCG (units)	Control		Intravenous HCG				After HCG							
					Day 1		Day 2		1st day		2nd day		3rd day		4th day	
			OHCS	KS	OHCS	KS	OHCS	KS	OHCS	KS	OHCS	KS	OHCS	KS	OHCS	KS
1	24	20,000	13.28	19.75	15.57	17.27	16.06	27.0	18.3	24.1	11.4	28.8				
2	22	20,000	4.4	10.1	7.6	12.5	3.8	15.6	3.5	16.7	5.1	20.2	2.7	15.4	2.9	8.1
3	48	20,000	<1.4*	12.5	7.4	13.9	3.9	14.8	4.2	13.7	<1.3	13.2	<1.1	17.7	<1.2	16.19
4	47	20,000	4.8	11.5	4.1	14.4	6.5	17.1	2.0	20.6	7.2	20.7	5.1	23.2	3.6	21.2
5	39	20,000	11.2	14.7	5.8	13.5	13.6	18.7	5.7	15.1	3.3	13.9	2.4	19.8	3.10	18.5
6	38	20,000	3.3	10.0	5.6	10.9	8.8	16.8	6.3	16.7	9.1	13.7	16.0	13.9	4.0	17.0
7	49	20,000	<1.2	15.1	5.5	19.0	3.3	19.5	2.5	18.0	4 8	22 6				
8	34	10,000	3.9	5.3	8.8	14.0	<3.6	37.4	10.6	31.8						
9	31	10,000	1.4	8.3	2.1	10.1	4.1	5.4	2.1	13.9	1.8	22.1				
3	48	10,000	2.0	10.4	2.8	17.8	1.9	12.5	2.0	14.8	3.0	16.0				
10	45	10,000	6.3	11.3	8.4	9.7	5.0	21.4	5.8	24.8	5.3	13.8				
4	47	10,000	1.9	11.1	3.3	14.8	6.3	20.1	2.7	14.8	4.1	19.2				
11	42	10,000	5.8	13.5	4.0	13.7	4.9	14.5	7.4	18.8	5.6	13.0				
12	30	10,000	4.2	8.9	2.4	20.8	2.9	10.3	3.8	13.23	3.1	7.9				
MEAN			4.64	11.61	5.94	14.45	6.05	17.57	5.50	18.34	5.00†	17.31†	4.26‡	18.0‡	2.96‡	16.2‡
S.E.			1.0	1.5	0.9	0.9	1.1	1.9	1.2	1.4	0.8	1.5	2.7	1.6	0.5	2.2

* When the specimens contained less than 1 mg. of 17-hydroxycorticoids per liter, the content is recorded as less than (<) the amount calculated on the basis of 1 mg. per liter

† Thirteen patients.

‡ Five patients.

significant at the 5 per cent level on the second day of the infusion. The increased levels of 17-ketosteroids continued for at least four days following the infusion, at which time the mean level was still elevated by 3 mg. per twenty-four hours above the control value in the 5 patients so studied. One patient was given 2 HCG tests with 20,000 units on separate occasions; there was no significant difference in the urinary steroid responses.

Combined 10,000 and 20,000 units (Table 2, Fig. 3). As there was no statistically significant difference between the values obtained with 10,000 units and 20,000 units of HCG, the 2 groups of data were combined for further analysis. When combined, there was a total of 14 tests on 12 patients. The results showed a mean rise in urinary 17-hydroxycorticosteroids of 1 mg. per twenty-four hours on the first day—a rise which remained unchanged on the second day of the infusion. This difference in the mean was not statistically significant. The 17-ketosteroid level rose above the control value by 3 mg. per twenty-four hours on the first day and by 6 mg. on the second day of HCG infusion. These differences were significant at the 5 per cent level for the first day and at the 1 per cent level for the second day. Again, the elevation continued above the control value for at least two days following the infusion. In the 2 patients who received both 10,000 and 20,000 units, there was no difference in the pattern of response.

5,000 units (Table 3, Fig. 2). Four patients received 5,000 units of HCG. In all there was an increase in urinary 17-hydroxycorticosteroids on the first day, and in 3 of the 4 there was an increase on both the first and second days of the infusion. The mean increase per twenty-four hours above the control level was 1.4 mg. on the first day and 1.3 mg. on the second day.

TABLE 3. URINARY 17-HYDROXYCORTICOSTEROID AND 17-KETOSTEROID RESPONSE TO INTRAVENOUS HCG (1,000 AND 5,000 UNITS) IN SUBJECTS WITH NORMAL ADRENOCORTICAL AND TESTICULAR FUNCTION

(mg. per 24 hrs.)

Patient No.	Age (yrs.)	Dose of HCG (units)	Control		Intravenous HCG				After HCG					
					Day 1		Day 2		1st day		2nd day		3rd day	
			OHCS	KS	OHCS	KS	OHCS	KS	OHCS	KS	OHCS	KS	OHCS	KS
13	49	1,000	1.38	23.96	2.03	39.20	7.27	29.19	13.63	31.23	3.14	12.82	7.02	12.31
16	35	1,000	1.49	16.55	1.46	18.30	3.80	20.20	8.58	13.40	1.90	15.10	3.70	12.30
17	41	1,000	1.64	9.54	3.55	29.72	8.14	14.59			5.30	13.30	4.74	20.81
Mean			*1.50*	*16.68*	*2.37*	*29.07*	*6.40*	*21.32*			*3.44*	*13.74*	*5.15*	*18.80*
15	58	5,000	4.90	12.97	5.41	13.88	3.37	9.01	7.72	16.84	11.88	8.06	6.27	9.47
16	35	5,000	1.57	15.00	5.15	24.90	2.05	13.10	1.50	16.70	1.60	18.50	1.40	17.20
13	49	5,000	1.47	20.50	1.62	23.18	3.72	18.88		25.03	1.54	29.71	1.86	29.74
18	38	5,000	12.29	9.50	12.68	15.62	16.14	11.30	4.25	13.03	10.38	13.50	14.15	14.49
Mean			*5.05*	*14.49*	*6.46*	*19.39*	*6.32*	*13.07*	*4.49*	*17.90*	*6.35*	*17.57*	*5.92*	*17.72*

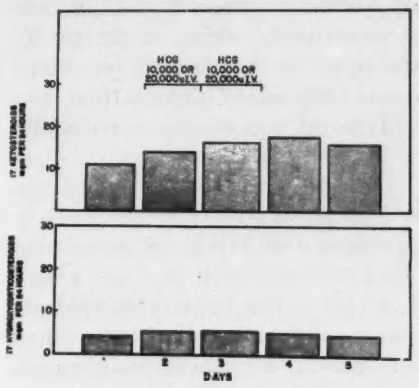

FIG. 3. The mean urinary steroid response to 10,000 or 20,000 units of HCG administered to 12 male subjects without known adrenocortical or testicular abnormality. The plotted mean levels represent 14 tests in 12 subjects.

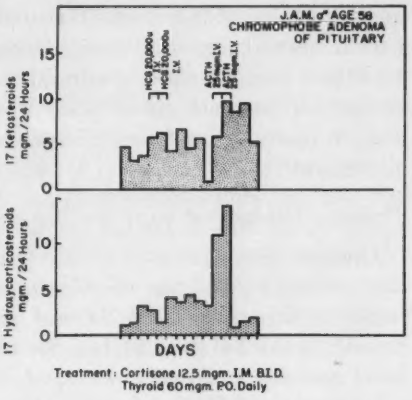
J.A.M. ♂ AGE 58
CHROMOPHOBE ADENOMA OF PITUITARY
17 Ketosteroids mgm./24 Hours
17 Hydroxycorticosteroids mgm./24 Hours
DAYS
Treatment: Cortisone 12.5 mgm. I.M. B.I.D.
Thyroid 60 mgm. P.O. Daily

FIG. 4. The urinary steroid response to intravenously administered ACTH and HCG in a male patient with panhypopituitarism being maintained on cortisone (12.5 mg. intramuscularly twice daily) and desiccated thyroid (60 mg. daily).

These differences were not statistically significant. In all of the patients there was an increase in urinary 17-ketosteroids on the first day (mean increase, 5.0 mg. per twenty-four hours). However, on the second day there was a slight increase in only 1 instance, and the mean level had decreased by 1 mg. per twenty-four hours (Table 3). It would appear that the response was not as consistent with 5,000 units as with 10,000 units of HCG.

1,000 units (Table 3). Three patients received 1,000 units of HCG. In 2 of the 3 there was an increase in urinary 17-hydroxycorticosteroids on the first day, and in all 3 there was an increase on the second day, the means being 1 mg. and 5 mg. per twenty-four hours, respectively. In all patients there was an increase in urinary 17-ketosteroids on the first and second days of the infusion. The mean increase per twenty-four hours was 12 mg. on the first day and 4 mg. on the second day. Although the data are too limited to draw definite conclusions, it would appear that 1,000 units elicited a pronounced response, which was greater than that obtained with 10,000 and 20,000 units in these subjects.

Supplementary Studies

Panhypopituitarism (Fig. 4)

One patient with panhypopituitarism resulting from a chromophobe adenoma (maintained on 12.5 mg. of cortisone intramuscularly and 60 mg. of desiccated thyroid orally each day) underwent an HCG test with 20,000

units and an ACTH test. The urinary steroid response to ACTH was within normal limits, although there was a one-day delay in the rise of 17-ketosteroids. Following administration of HCG, there was no consistent change in the excretion of 17-hydroxycorticosteroids. Although there was a slight increase in urinary 17-ketosteroid excretion, it was not statistically significant.

Primary hypogonadism (Figs. 5, 6 and 7)

One patient with primary hypothyroidism and hypogonadism, being maintained on 120 mg. of desiccated thyroid orally each day, was tested with 20,000 units of HCG and with ACTH. The urinary steroid response to ACTH was within normal limits, although the 17-ketosteroid level was not strikingly increased. There was no obvious response to administration of HCG (Fig. 5). One patient (C.J., Fig. 6) who had had a bilateral orchiectomy and was being maintained on 25 mg. of chlorotri-

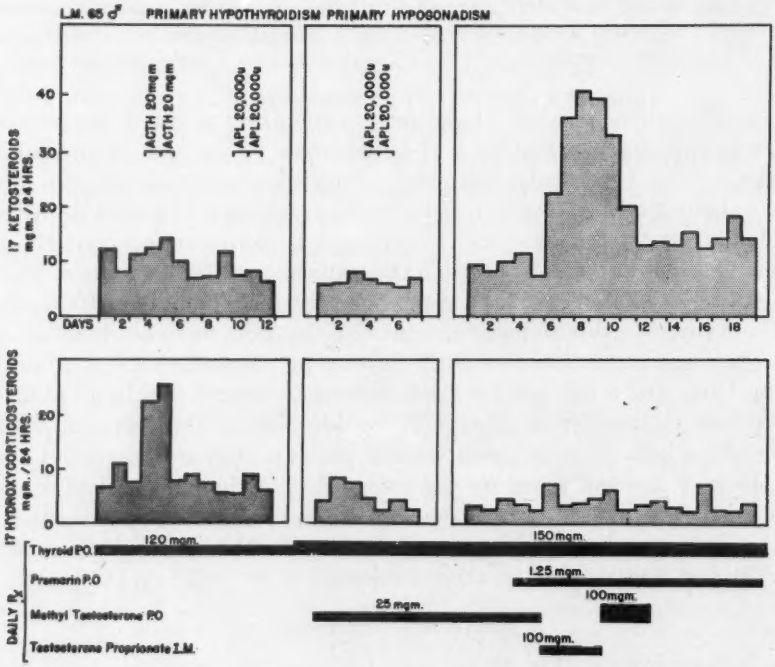

FIG. 5. The urinary steroid response to intravenously administered ACTH and HCG in a male patient with primary hypothyroidism and hypogonadism. The effect of conjugated estrogens (Premarin), methyltestosterone and testosterone propionate on these steroids is also illustrated.

anisene (TACE)[6] orally each day, had a normal urinary steroid response to ACTH. Following administration of HCG there was no significant change in urinary 17-hydroxycorticosteroids or 17-ketosteroids, although an elevation in the excretion of 17-ketosteroids was noted the day following the infusion, with a prompt return to baseline levels on the next day. A third patient (B.S., Fig. 7) was given 160 mg. of ACTH intramuscularly (40 units every six hours) on the first day; nothing on the following day; and a repetition of the intramuscular ACTH on the third day. There was a normal response in both 17-ketosteroid and 17-hydroxycorticosteroid excretion. For an HCG test this same patient received 20,000 units of HCG intravenously on the first day; nothing on the following day; and 20,000 units of HCG intramuscularly on the third day. Again there was no significant change in urinary 17-hydroxycorticosteroid excretion, whereas there was a definite increase in 17-ketosteroid excretion on the second day after administration of HCG, with a rapid rebound-decrease on the day following.

This increase in the 17-ketosteroid levels following administration of HCG to Patients C.J. and B.S. was unlike that seen following administration of either ACTH or HCG to normal subjects. The interpretation can be only speculative.

Addison's disease (*Fig. 8*)

One patient with classic Addison's disease was studied while being maintained on 25 mg. of cortisone intramuscularly per day. As anticipated, there was no significant increase in either 17-hydroxycorticosteroid or 17-ketosteroid excretion following the administration of ACTH. The administration of 20,000 units of HCG resulted in a stepwise and sustained increase in urinary 17-ketosteroids, the maximum level being reached on the day following the infusion. There was also a suggestive increase in urinary 17-hydroxycorticosteroids. As the rise in 17-hydroxycorticosteroids as well as in 17-ketosteroids was sustained, it was felt that at least part of the increment might be explained on the basis of the effect of the intramuscular cortisone therapy which was begun two days prior to the study. The rise in 17-ketosteroid excretion, however, was of a greater magnitude than would be expected due to cortisone alone.

Bilateral adrenalectomy and orchiectomy (*Fig. 9*)

Subject W.G., who had undergone bilateral orchiectomy and adrenalectomy for carcinoma of the prostate, was being maintained on a daily dose

[6] We are indebted to Doctors Raymond C. Pogge and R. H. McMaster, Department of Medical Research, The William S. Merrell Co., for the generous supply of TACE used in these studies.

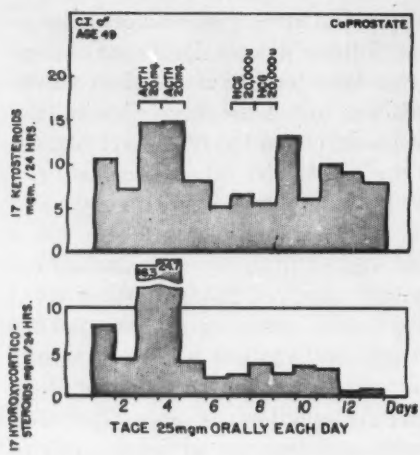

FIG. 6. The urinary steroid response to intravenously administered ACTH and HCG in a gonadectomized male patient.

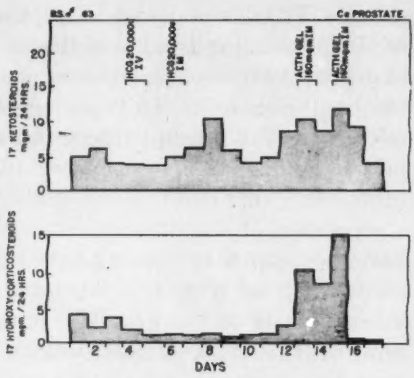

FIG. 7. The urinary steroid response to ACTH and HCG in a gonadectomized male patient. Note the different routes and timing of hormone administration.

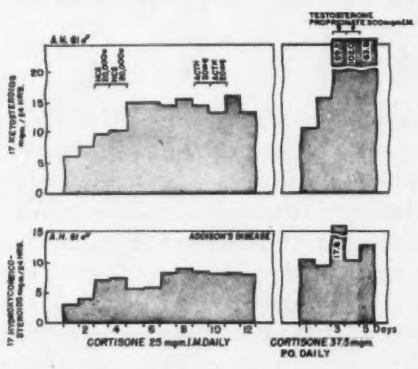

FIG. 8. The urinary steroid response to intravenously administered ACTH and HCG in a male patient with Addison's disease. The effect of massive doses of testosterone propionate on the urinary steroid levels is also shown.

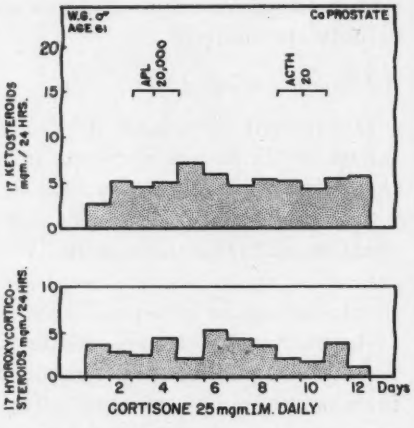

FIG. 9. The urinary steroid response to intravenously administered HCG and ACTH in a gonadectomized, adrenalectomized male subject.

of 25 mg. of cortisone intramuscularly. No increase in urinary 17-hydroxycorticosteroid or 17-ketosteroid excretion was seen following the intravenous administration of ACTH or HCG. It would therefore appear unlikely that the urinary steroid changes observed in the other subjects would be the result of nonspecific renal or circulatory effects.

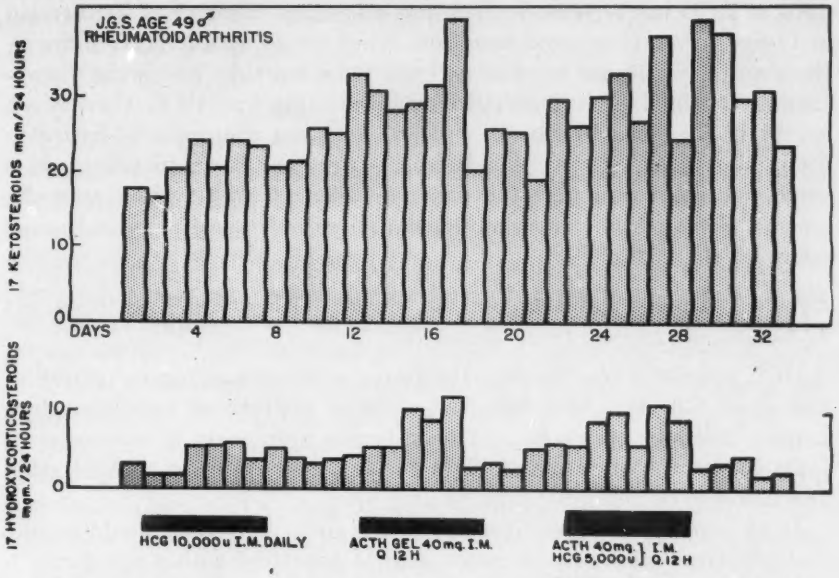

FIG. 10. The urinary steroid response to simultaneously administered ACTH and HCG in a subject with no known testicular or adrenal disease.

Possible synergistic or antagonistic action of simultaneously administered ACTH and HCG (Fig. 10)

Patient J.G.S., who had no known abnormalities of adrenocortical or testicular function, was given 10,000 units of HCG intramuscularly daily for six days, during which time there was a rise in urinary 17-ketosteroid excretion which was sustained after discontinuing HCG. There was no consistent change in urinary 17-hydroxycorticosteroid excretion. Following the administration of 40 mg. of ACTH-gel intramuscularly every twelve hours for six days, there was a steady rise in both urinary 17-ketosteroid and 17-hydroxycorticosteroid excretion, which promptly returned to baseline values following discontinuance of ACTH. With the simultaneous administration of ACTH and HCG, the response was similar to that with ACTH alone, except for the sustained elevation of 17-ketosteroid excretion. The failure to demonstrate a greater increase in urinary 17-ketosteroids when both tropic hormones were given simultaneously than when given separately, is noteworthy (32, 33).

Effect of inhibition of ACTH on the response to HCG

One patient with normal adrenocortical and testicular function was given fluorohydrocortisone, 1 mg. daily for five days. When tested with 20,000

units of HCG intravenously, there was no change in either 17-ketosteroid or 17-hydroxycorticosteroid excretion. When tested with ACTH, however, there was a significant increase in both these steroids. Following discontinuance of the fluorohydrocortisone and retesting with HCG, there was a rise in 17-ketosteroid excretion but no consistent change in 17-hydroxycorticosteroid excretion. Allen Goldfein (personal communication), in a similar study, found that the urinary 17-ketosteroids, which were decreased during administration of fluorohydrocortisone, rose following administration of HCG.

Effect of estrogens and androgens on urinary 17-hydroxycorticosteroid and 17-ketosteroid excretion (Figs. 5 and 8)

HCG is known to stimulate the testes to secrete estrogens as well as androgens (19, 34). Therefore, the effect of conjugated estrogens (Premarin), methyltestosterone and testosterone propionate in various combinations on the excretion of 17-hydroxycorticosteroids was investigated and found to be negligible (Fig. 5).

In an attempt to determine whether or not testosterone would modify 17-hydroxycorticosteroid excretion (35) in a patient with a constant exogenous source of cortisone (Fig. 8), testosterone propionate in a dosage of 300 mg. daily was administered intramuscularly on two consecutive days to a patient with Addison's disease receiving 37.5 mg. of cortisone daily by mouth. There was a marked rise in the urinary 17-ketosteroid excretion, as would be expected. There was also a rise in urinary 17-hydroxycorticosteroid excretion on the first day of testosterone therapy which, however, was not sustained when testosterone was continued. It is concluded, therefore, that stimulation of testicular androgens or estrogens would not modify any possible changes in urinary 17-hydroxycorticosteroids resulting from administration of HCG.

Reproducibility and specificity of response

One normal subject (No. 2) was given 20,000 units of HCG on 2 different occasions and similar responses were obtained. Another patient (No. 10) was given an intravenous injection of 1,000 ml. of 5 per cent dextrose and water, without added hormone, over eight hours; no change was noted in either 17-hydroxycorticosteroid or 17-ketosteroid excretion. Another patient was given an intravenous infusion of 400 ml. of gamma globulin (as a nonspecific protein substance) over eight hours; no change was observed in either 17-ketosteroid or 17-hydroxycorticosteroid excretion.

Further evidence for specificity of response is provided by the lack of a response when deactivated HCG (heated at 70°C. for twelve hours) was

infused into normal persons who had previously been shown to be responsive to unmodified HCG.

DISCUSSION

By comparing, in the same subjects, the response of the urinary total 17-hydroxycorticosteroid and 17-ketosteroid levels to the slow intravenous administration of ACTH and HCG, the difference between these responses becomes apparent. In subjects with clinically normal adrenocortical and testicular function, the intravenous administration of ACTH over an eight-hour period on two consecutive days resulted in a prompt increase in the excretion of both types of steroid on the first day and a further increase on the second day of administration, with a relatively greater increase in the 17-hydroxycorticosteroid levels on both days. On the day following administration of ACTH, the urinary excretion of both these steroids returned to the respective baseline levels.

HCG administered in a similar manner to the same patients elicited a significant increase in urinary 17-ketosteroid excretion beginning on the first day of the infusion, continuing on the second day and reaching a maximum on the day following the infusion. Although the levels began to decrease on the second day after the infusion, they remained elevated in some subjects for as long as five to six days. Urinary 17-hydroxycorticosteroid excretion was increased in a few subjects, although there was no significant increase in the mean level for the entire group.

Insofar as adrenocortical activation can be inferred from an increase in total urinary 17-hydroxycorticosteroid excretion, human chorionic gonadotropin does not stimulate the adrenal cortices. This does not exclude the possibility that adrenal androgens alone are being stimulated by this material. However, the obvious difference in the patterns of increased urinary 17-ketosteroid excretion following the ACTH test as compared with the HCG test suggests that HCG does not stimulate the adrenal cortex in a manner similar to ACTH. Results of studies on a 75-year-old woman with the adrenogenital syndrome add further support to the concept that adrenocortical androgenic tissue and pituitary gonadotropin do not have the usual reciprocal relationship that exists between a target gland and its tropic hormone. In this patient, urinary 17-ketosteroid excretion was high (65 mg. per twenty-four hours) because of congenital adrenocortical hyperplasia, but urinary gonadotropin excretion was also high (1,388 mouse units per twenty-four hours) because of the menopausal syndrome. If the reciprocal relationship between adrenal androgens and the pituitary were similar to that between gonadal androgens and the pituitary, one would have expected an inhibition of pituitary gonadotropin as

a result of the markedly elevated adrenal androgens. In addition, the lack of response to HCG in patients with primary hypogonadism also suggests that HCG stimulates only testicular tissue.

As a significant increase in 17-ketosteroid excretion was seen during administration of 10,000 and 20,000 units of HCG in normal subjects, one could theoretically use this procedure as a rapid test of testicular interstitial-cell function (36). However, in the one patient with panhypopituitarism who was studied, urinary 17-ketosteroids increased only slightly following administration of HCG. With this test alone it would be difficult to distinguish between primary and secondary hypogonadism in this patient. It is possible, however, that more prolonged stimulation with HCG would result in a more pronounced increase in urinary 17-ketosteroid excretion. A negative response to HCG, therefore, could not be definitively interpreted, whereas a positive response would be diagnostically helpful in this regard. It is apparent that patients with primary hypogonadism do not respond to intravenous HCG by an increase in urinary steroids.

With the technics employed, 10,000 units of HCG administered intravenously elicited a maximal response; 5,000 units did not elicit as definite nor as sustained a response as with 10,000 or 20,000 units (Fig. 2). The response to 1,000 units in 3 patients was more marked than with any of the other doses. Whether or not this pattern of response would be borne out by the findings in a larger study cannot be decided at this time.

Failure to obtain changes in urinary steroid excretion following the administration of ACTH and HCG to a patient who had undergone bilateral orchiectomy and adrenalectomy and was being maintained on cortisone, provides evidence against the concept that nonspecific renal or circulatory effects were the cause of the changes noted in the normal subjects. Failure to obtain changes in the urinary corticoid excretion following the administration of estrogens, androgens, gamma globulin or deactivated HCG would also suggest that the urinary steroid changes observed were specific for HCG or ACTH and were unmodified by other responses such as HCG-stimulated secretion of estrogen or androgen by testicular interstitial cells, or nonspecific responses to protein substances.

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REFERENCES

1. Zondek, B., and Sulman, F.: Mechanism of action and metabolism of gonadotropic hormones in the organism, *Vitamins & Hormones* **3**: 297, 1945.
2. Reifenstein, E. C., Jr.; Forbes, A. P.; Albright, F.; Donaldson, E., and Carroll E.: The effect of methyltestosterone on urinary 17-ketosteroids of adrenal origin, *J. Clin. Invest.* **24**: 416, 1945.

3. Cerasuolo, G.: Urinary steroids in ovariectomized woman after administration of chorionic gonadotropins, *Arch. ostet. e ginec.* **59:** 376, 1954.
4. Donato, L.; Turchetti, G., and Zacutti, A.: Uptake of radiophosphorus by the adrenals in male rats following stimulation with chorionic gonadotropins and ACTH, *Folia endocrinol.* **8:** 569, 1955.
5. Heller, C. G., and Nelson, W. O.: The testis-pituitary relationship in man, *Recent Progr. Hormone Res.* **3:** 229, 1948.
6. Harrison, J. H.; Leman, C. B.; Munson, P. L., and Laidlaw, J. C.: Androgen gonadotrophin and steroid excretion in man before and after castration and adrenalectomy, *J. Urol.* **73:** 580, 1955.
7. Landing, B. H., and Gold, E.: The occurrence and significance of Leydig cell proliferation in familial adrenal cortical hyperplasia, *J. Clin. Endocrinol.* **11:** 1436, 1951.
8. Moracci, E.: Effects of chorionic gonadotropin in senescence in women and the problem of sexual endocrine activity of the adrenal cortex, *Minerva ginec.* **5:** 421, 1953.
9. Plate, W. P.: Stimulating effect of chorionic gonadotrophin on adrenal cortex, *Acta endocrinol.* **11:** 119, 1952.
10. Borell, U.: The effect of large doses of human chorionic gonadotrophin on the excretion of neutral 17-ketosteroids in women, *Acta endocrinol.* **17:** 13, 1954.
11. Borth, R.; Gsell, M., and de Watteville, H.: Urinary steroids and vaginal smear in ovariectomized women treated with chorionic gonadotrophin, *Acta endocrinol.* **14:** 316, 1953.
12. Carreyett, R. A.; Golla, Y. M. L., and Reiss, M.: Action of gonadotrophic hormone and of pituitary corticotrophic hormone on cholesterol content of adrenals, *J. Physiol.* **104:** 210, 1945.
13. Ceresa, F., and Rubino, G. F.: Testicular reaction to experimental stimulation with chorionic gonadotropin in male hypogenitalism; variations of 17-ketosteroids in urine with respect to stimulation test, *Minerva med.* (suppl.) **2:** 1115, 1950.
14. Decourt, J.; Jayle, M. F.; Michard, J. P., and Ramon, C. G.: Panhypopituitarism; effects of chorionic gonadotropin and ACTH on the urinary elimination of different categories of steroids, *Ann. d'endocrinol.* **14:** 892, 1953.
15. Diczfalusy, E.; Holmgren, H., and Westman, A.: Action of human chorionic gonadotrophin preparations on adrenals and testicles of hypophysectomized immature rats, *Acta endocrinol.* **5:** 43, 1950.
16. Decio, R.: The effects of gonadotropic hormones in surgically castrated women, *Acta endocrinol.* **19:** 185, 1955.
17. Ferin, J., and Devis, R.: The effects of chorionic gonadotropin in an ovariectomized woman, *Ann. d'endocrinol.* **10:** 563, 1949.
18. Kyle, L. H., and O'Donovan, T. F.: Pituitary hypogonadism with adrenal androgen deficiency: the effect of chorionic gonadotropin on 17-ketosteroid excretion, *J. Clin. Endocrinol.* **10:** 330, 1950.
19. Maddock, W. O., and Nelson, W. O.: The effects of chorionic gonadotropin in adult men: increased estrogen and 17-ketosteroid excretion, gynecomastia, Leydig cell stimulation and seminiferous tubule damage, *J. Clin. Endocrinol. & Metab.* **12:** 985, 1952.
20. Segaloff, A., and Parson, W.: Hypogonadotropic eunuchoidism: report of case with failure to respond to chorionic gonadotropic hormone due to antihormones, *J. Clin. Endocrinol.* **7:** 130, 1947.
21. Silvestrini, F.; Pasargiklian, E.; Litta, R.; Melloni, G., and Salvetti, V.: Influence of chorionic gonadotropins on adrenal cortex function, *Rass. fisiopat. clin.* **25:** 261, 1953.

22. Tellez, A. R., Yanez, R. A., and Hamuy, Y.: Effect of human chorionic gonadotropins on excretion of 17-ketosteroids, *Rev. méd. Valparaiso* **5**: 274, 1952.
23. Wells, L. J.: Effects of injections of equine gonadotropin upon gonads and adrenals of fetal rats, *Proc. Soc. Exper. Biol. & Med.* **62**: 250, 1946.
24. Jenkins, D.; Forsham, P. H.; Laidlaw, J. C.; Reddy, W. J., and Thorn, G. W.: Use of ACTH in the diagnosis of adrenal cortical insufficiency, *Am. J. Med.* **18**: 3, 1955.
25. Drekter, I. J.; Heisler, A.; Scism, G. R.; Stern, S.; Pearson, S., and McGavack, T. H.: The determination of urinary steroids. I. The preparation of pigment-free extracts and a simplified procedure for the estimation of total 17-ketosteroids, *J. Clin. Endocrinol. & Metab.* **12**: 55, 1952.
26. Holtorff, A. F., and Koch, F. C.: The colorimetric estimation of 17-ketosteroids and their application to urine extracts, *J. Biol. Chem.* **135**: 377, 1940.
27. Reddy, W. J.: Modification of the Reddy-Jenkins-Thorn method for estimation of 17-hydroxycorticoids in urine, *Metabolism* **3**: 489, 1954.
28. Lambert, G. F.: The determination of creatine and creatinine, *J. Biol. Chem.* **161**: 679, 1945.
29. Sayers, M. A.; Sayers, G., and Woodbury, L. A.: The assay of adrenocorticotrophic hormone by the adrenal ascorbic acid-depletion method, *Endocrinology* **42**: 379, 1948.
30. Opsahl, J. C.; Long, C. N. H., and Fry, E. G.: Chorionic gonadotropin, ACTH (adrenocorticotropic hormone) and adrenal-hyaluronidase relationship, *Yale J. Biol. & Med.* **23**: 399, 1951.
31. Madsen, V.; Pedersen-Bjergaard, K.; Roholt, K., and Tonnesen, M.: Antigen and pyrogen content in commercial chorionic gonadotropin preparations, with reference to their clinical application, *Acta endocrinol.* **2**: 140, 1949.
32. Clayton, B. E., and Hammant, J. E.: Modification of response to adrenocorticotrophic hormone by the simultaneous administration of gonadotrophic hormones, *Nature* **176**: 401, 1955.
33. Smith, E. K.: Interrelationships of anterior pituitary and adrenal cortex in the rat ovarian hyperemia reaction, *Endocrinology* **56**: 567, 1955.
34. Conti, C.; Marinosci, A., and Mazzuoli, G. F.: Estrogenic hormones in man. I. Effect of chorionic gonadotropin and pituitary corticotropin on urinary excretion of estrogens and 17-ketosteroids in the normal male, *Folia endocrinol.* **8**: 383, 1955.
35. Venning, E. H., and Browne, J. S. L.: Effect of testosterone on the excretion of glycogenic corticoids, *J. Clin. Endocrinol.* **7**: 729, 1947.
36. Botella Llusia, J.: Curve of 17-ketosteroids following injection of chorionic gonadotropin as sex adrenal function test, *Obst. y ginec. latino-am.* **11**: 235, 1953.

CLINICAL EFFECT OF HUMAN PITUITARY FOLLICLE-STIMULATING HORMONE (FSH)*

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ABSTRACT

A partially purified follicle-stimulating hormone preparation (human pituitary FSH) has been obtained from human pituitaries. The ovarian response to this preparation was studied in 7 amenorrheic women. The effect of human chorionic gonadotropin (HCG) was studied in addition.

In 4 patients exhibiting no endometrial activity or only slight proliferation, HCG alone did not induce ovulation and had no effect on the size of the uterus, on the endometrium, or on the urinary excretion of estrogen and pregnanediol. In 2 patients showing endometrial proliferation, the administration of HCG alone was followed by ovulation, a secretory transformation of the endometrium, and a marked increase in urinary pregnanediol excretion.

The administration of human pituitary FSH alone to 2 patients resulted in an increase in the size of the uterine cavity, in polycystic enlargement of the ovaries, and in a pronounced increase in urinary estrogen output.

Treatment with human pituitary FSH followed by HCG produced in all patients polycystic enlargement of the ovaries, ovulation in 4 out of 5, and a secretory transformation of the endometrium in 3 out of these 5 patients. Ovulation was accompanied by a marked increase in the urinary excretion of both estrogen and pregnanediol.

FOLLICLE-STIMULATING hormone (FSH) has been purified from sheep (1) and hog (2) pituitaries. Such preparations possess a high degree of purity and have been tried in clinical studies by several investigators, but with inconclusive results. Some investigators report definite hormonal effects, whereas in the opinion of others the results are discouraging. The reasons for the inadequate clinical effect of such preparations may be sought in 1) species differences, 2) differences in hormonal requirement, or 3) the formation of antihormone which, following repeated administration, might interfere not only with the action of exogenous gonadotropins but also with that of the endogenous ones (3).

It has been demonstrated by Bahn *et al.* (4) that homogenates of human

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anterior pituitaries possess FSH activity when administered to hypophysectomized immature female rats. Since the evidence available at present (5) seems to indicate that gonadotropin extracted from human urine has no ability to provoke antihormone formation in clinical experiments, an attempt was made to purify follicle-stimulating hormone from human pituitaries. The data presented in this paper indicate that it is possible to obtain human pituitary follicle-stimulating hormone (hereafter called human pituitary FSH) from autopsy material, and that such preparations exhibit a marked hormonal effect when tested in human beings.

SUJBECTS AND PROCEDURE

The human pituitary FSH preparation was tested in 7 young amenorrheic women. The relevant anamnestic data are listed in Table 1.

Two of the 7 patients had primary amenorrhea and 5 had secondary amenorrhea, of at least two years' duration. Urinary gonadotropin excretion was low or normal. None of the patients had been treated previously with pregnant mare's serum gonadotropin (PMS) or with any other gonadotropic preparation.

Scheme of treatment. Since it has been reported by several investigators that a luteinizing factor, such as human chorionic gonadotropin (HCG), by itself may induce ovulation and endometrial changes in amenorrheic women, the effect of HCG ("Gonadex-Leo," manufactured by AB Leo, Hälsingborg, Sweden) was studied in each patient one to two months prior to the start of treatment with human pituitary FSH. The dosage was 6,000 I.U. given intramuscularly daily for at least six days. Human pituitary FSH was injected intramuscularly in daily doses of 10 to 30 mg. It was administered either alone or in combination with 6,000 I.U. of HCG daily. No untoward effects, such as fever or local reaction at the site of injection, were observed.

Assessment of clinical effect. In the assessment of the clinical effect of human pituitary FSH the following procedures were employed: measurement of the uterine cavity, palpation of the ovaries, inspection of the ovaries by culdoscopy, histologic picture of the

TABLE 1. SOME CLINICAL AND LABORATORY FINDINGS IN 7 WOMEN WITH AMENORRHEA, BEFORE TREATMENT

Patient No.	Initials	Age (yrs.)	Type of amenorrhea	Duration of amenorrhea (yrs.)	Size of uterine cavity (cm.)	Endometrial activity*	Urinary gonadotropin† (M.U./24 hrs.)	Comments
1)	G.K.	22	Primary	—	5	0	<13	
2)	M.H.	25	Primary	—	4	0	>6.5<13	
3)	B.Ö.	29	Secondary	4	6	0	<6.5	Endometrial damage (?)
4)	L.F.	21	Secondary	2	6	P (weak)‡	>13<53	
5)	E.G.	26	Secondary	6	7	P (medium)	<6.5	
6)	I.F.	22	Secondary	5	8	P (medium)	>6.5<13	
7)	A.S.	25	Secondary	3	7	P (strong)	>6.5<13	Stein-Leventhal syndrome (?). Two menstrual-like bleedings during the previous 3 years.

* Repeated findings.

† Mouse uterus test. A "mouse unit" (M.U.) corresponds roughly to the activity of 0.3 to 0.6 mg. of HMG-20 standard.

‡ P = proliferative.

endometrium, and determination of urinary estrone, estradiol-17β, estriol, pregnanediol, 17-ketosteroids and 17-hydroxycorticosteroids.

METHODS

Preparation of human pituitary FSH

The purification of human pituitary FSH is based on the assumption that (as with sheep FSH) it is the principal pituitary hormone soluble in ammonium sulphate solution above 50 per cent saturation (1). Human pituitaries obtained at autopsy were frozen and lyophilized. The dried glands were cut into small pieces, homogenized, and extracted in cold CaO solution at pH 9.3 under continuous stirring for at least six hours. Approximately a 10-ml. volume of CaO solution was used per 100 mg. of dried pituitary. After centrifugation, the clear supernatant liquid was slowly brought to 55 per cent saturation by the addition of a saturated solution of ammonium sulphate. The solution was allowed to stand overnight in a cold room (4° C.) and then centrifuged. The precipitate was discarded and the clear supernatant layer was brought to 75 per cent saturation by the addition of solid ammonium sulphate. The material precipitating between 55 and 75 per cent saturation with ammonium sulphate was collected by centrifugation, dissolved in water, dialyzed and lyophilized. This product, provisionally designed as human pituitary FSH, was used in the clinical trials.

Potency of human pituitary FSH when assayed in immature rats

The human pituitary FSH was assayed against the provisional human menopausal gonadotropin (HMG-20) standard preparation. This urinary standard possesses both FSH- and LH-activity (6). The uterine weight method was used as described previously (7), the ovarian weight method was carried out according to Albert (8), and the ovarian augmentation test according to Steelman and Pohley (9), with the exception that the amount of HCG used was only 10 I.U. The method based on the enlargement of the ventral prostate in hypophysectomized rats was carried out as described by Greep *et al.* (10), using the modification introduced by Loraine and Brown (11). With the exception of the ventral prostate method, in all assays littermate control was employed. The statistical methods used for calculating the relevant parameters have been described in detail elsewhere (12, 13). All assays satisfied the recognized criteria of validity. The results are summarized in Table 2.

It may be seen that, on a weight basis, the partially purified human pituitary FSH was 30 to 50 times more potent than the HMG-20 standard preparation, when assayed by various methods measuring total gonadotropic or FSH activities. On the other hand.

TABLE 2. POTENCY OF PARTIALLY PURIFIED HUMAN PITUITARY FSH WHEN ASSAYED AGAINST HUMAN MENOPAUSAL GONADOTROPIN (HMG-20) STANDARD IN IMMATURE RATS

Type of immature rat	Index of response	No. of rats per assay	Potency of human pit. FSH (per mg.) *vs.* HMG-20	Fiducial limits of error (P = 0.95)	Index of precision (λ)	Index of discrimination
Intact female	Uterine weight	28	47.7 ×	41.4–54.4	0.07	—
Intact female	Ovarian weight	38	39.4 ×	27.3–50.0	0.13	1.21
HCG-treated female	Ovarian weight	36	28.3 ×	22.3–35.7	0.14	1.69
Hypophysectomized male	Ventral prostate weight	14	5.4 ×	2.8– 9.5	0.14	8.85

in the ventral prostate test, which is considered to be specific for LH-activity, the human pituitary FSH was only approximately 5 times as potent as HMG-20. Gaddum's (14) index of discrimination was also applied to the data, as shown in the last column of Table 2. This index is calculated by dividing the potency ratio obtained in one test by the corresponding figure in another. This index is close to unity when 2 substances possessing identical biologic activity are assayed. When the uterine weight method was employed as the basis for this comparison, only the value obtained by the ventral prostate method showed a greater divergence from unity. This would seem to indicate that the partially purified human pituitary FSH was a richer source of FSH- than of LH-activity, when compared to urinary menopausal gonadotropin. The partially purified human pituitary FSH was also assayed for growth hormone (GH)-activity, using the tibial test of Evans *et al.* (15) in hypophysectomized rats. Only traces of GH-activity were found.

The fraction precipitating at 55 per cent ammonium sulphate saturation (see section on preparation of human FSH) was also tested at the 1-mg. dose level for FSH-activity in hypophysectomized immature female rats. No follicular growth was observed, but the repair of interstitial wheel cells indicated the presence of luteinizing hormone (LH)-activity. A Steelman-Pohley test (9) was also carried out at the same dose level, with negative results. Thus, it would appear from these data that it is possible to obtain from human pituitaries partially purified fractions exhibiting predominantly FSH- and LH-activities, respectively.

Assessment of endometrial biopsy specimens

All patients were followed by serial endometrial biopsies. The endometrium was recorded as being:

Atrophic (A) *i.e.*, no endometrial material or only a thin endometrium with small inactive glands could be obtained.

Proliferative (P), graded as slight ($\overline{P}$), moderate (P) and marked ($\underline{P}$).

Secretory (S), sometimes combined with decidual reaction ($\underline{S}$).

Each biopsy specimen from the same patient was taken from a different part of the uterine cavity in order to avoid areas of tissue damage from previous biopsies.

Urinary steroid assays

Estrogen assays were restricted to the estimation of the 3 "classic" estrogens—estrone, estradiol-17β and estriol. The method of Brown (16) was used, with slight modifications as described by Diczfalusy *et al.* (17) and Brown *et al.* (18). The term "estrogen" will be used in this paper in a loose sense to denote estrone, estradiol-17β and estriol.

Pregnanediol was estimated according to the method of Klopper *et al.* (19), with the exception that the color correction equation of Allen (20) was used. The evidence in favor of this modification has been presented elsewhere (21).

17-Ketosteroids (17-KS) were estimated according to the micromethod of Vestergaard (22), with certain modifications, including the use of the Allen (20) formula.

Corticosteroids were assayed as 17-hydroxycorticosteroids (17-OHCS), using the method described by Appleby *et al.* (23) as modified by Birke *et al.* (24). Thus, the term "urinary corticosteroids" will be used to denote all C_{21}-steroids possessing a 17-hydroxyl group, including compounds with the 17:20-glycol, 17:20-ketol, 17:20:21-triol and dihydroxyacetone side-chain.

RESULTS

Case 1

The results obtained in this patient are shown in Figure 1. It may be seen that HCG alone had no effect on the endometrium or on the urinary excretion of estrogen and pregnanediol.

Human pituitary FSH administered for six days induced a polycystic enlargement of the ovaries and a significant rise in estrogen excretion, without any effect on pregnanediol excretion. When human pituitary FSH was supplemented with HCG, the urinary estrogen excretion reached levels usually seen only in pregnancy. The rise in estrogen excretion was accompanied by a marked rise in urinary pregnanediol and also in urinary corticosteroid excretion. The endometrium changed from a completely atrophic state to a secretory phase, with an almost decidua-like histologic picture, as shown in Figure 2.

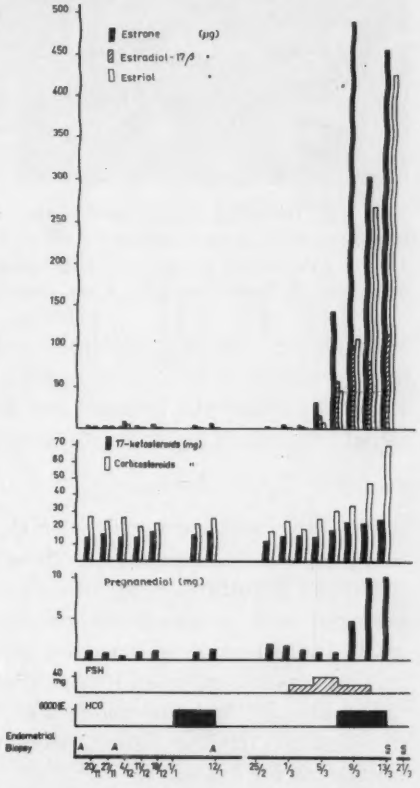

FIG. 1. Endometrial activity and 48-hour urinary excretion of estrogen, 17-ketosteroids, corticosteroids and pregnanediol in Case 1 during the different phases of the treatment. The dose and duration of treatment by human chorionic gonadotropin (HCG) and by human pituitary FSH are also indicated. In this and subsequent figures, biopsy findings are designated as follows: A = atrophic, P = proliferative, and S = secretory (see text for modifications).

FIG. 2. Histologic appearance of the endometrium in Case 1 following combined treatment with human pituitary FSH and human chorionic gonadotropin (HCG). **A.** Rather wide, coiled glands with large subnuclear vacuoles; only a few vacuoles in the inner zone. **B.** Eight days later. The interstitial cells are enlarged and almost decidua-like.

Culdoscopy revealed enlarged polycystic ovaries (the left one with a diameter of at least 10 cm.) and a fresh ovulation in the right ovary. Eight days later the enlargement disappeared and the patient had menstrual bleeding of six days' duration.

Case 2

HCG by itself had no effect on the endometrium or on urinary estrogen and pregnanediol excretion, as shown in Figure 3.

Human pituitary FSH administered alone had no effect on urinary estrogen and pregnanediol excretion, but when it was followed by HCG the excretion of estrogen increased significantly. Pregnanediol excretion was not affected in this case.

Culdoscopy revealed moderately enlarged polycystic ovaries without any sign of a fresh ovulation. Menstrual bleeding occurred eight days later and lasted for five days.

Case 3

HCG had no major effect on urinary estrogen and pregnanediol excretion, as shown in Figure 4.

Human pituitary FSH caused a minor but significant increase in the estrogen output. Combined treatment with human pituitary FSH+HCG resulted in greatly increased estrogen and pregnanediol excretion accom-

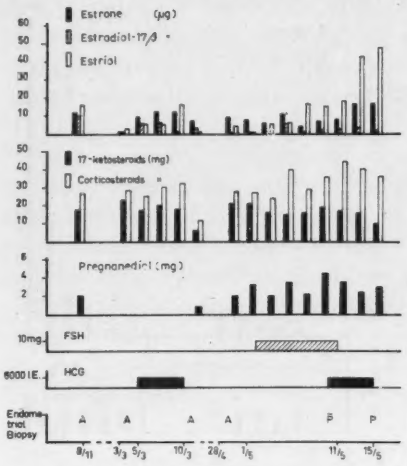

FIG. 3. Steroid excretion (48 hours) and endometrial activity in Case 2, during treatment with HCG and h.pit. FSH.

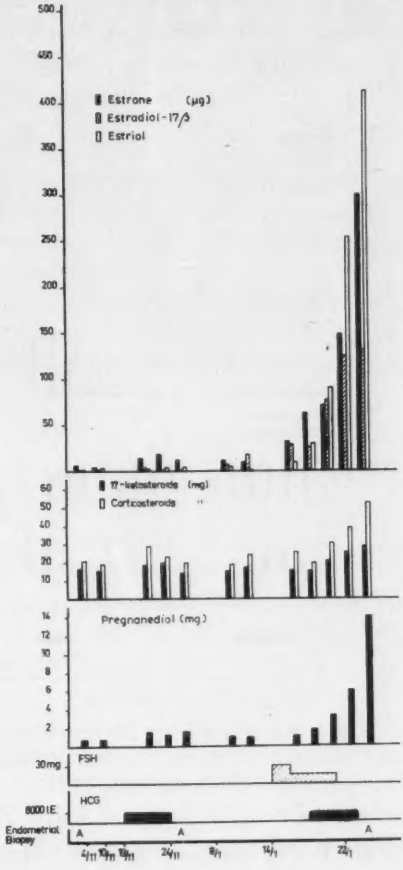

FIG. 4. Steroid excretion (48 hours) and endometrial activity in Case 3 during treatment with HCG and h.pit. FSH.

panied by a rise in urinary corticosteroid excretion.

Culdoscopy showed polycystic enlargement of the ovaries and a fresh ovulation. There were no signs of activation in the endometrium, but the size of the uterine cavity increased by at least 2 cm. Five days later scanty menstrual bleeding occurred, which lasted for five days.

Case 4

As shown in Figure 5, HCG had no effect on urinary pregnanediol excretion and only a slight effect on urinary estrogen excretion or on the activity of the endometrium.

Human FSH by itself had no effect on pregnanediol excretion and but

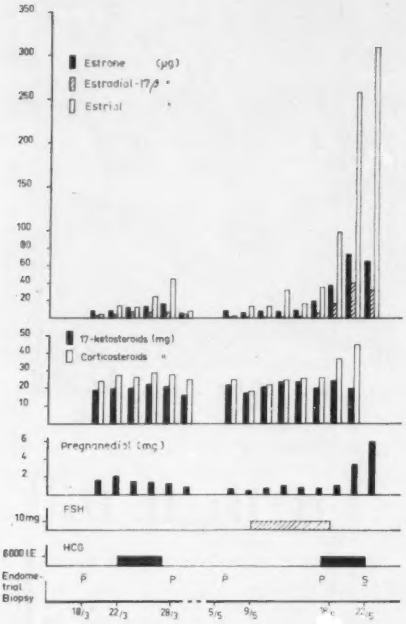

FIG. 5. Steroid excretion (48 hours) and endometrial activity in Case 4 during treatment with HCG and h.pit. FSH.

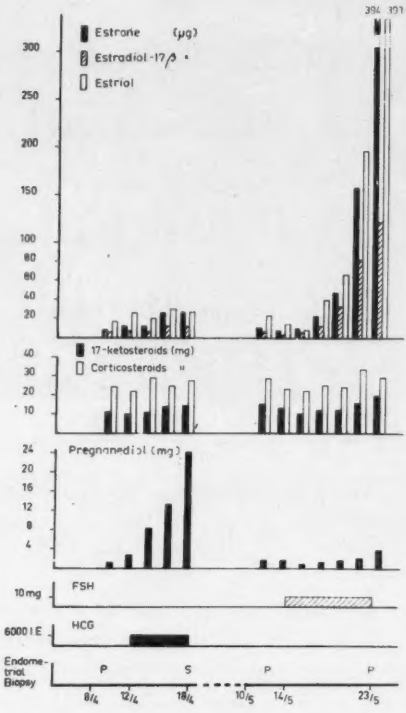

FIG. 6. Steroid excretion (48 hours) and endometrial activity in Case 5 during treatment with HCG and h.pit. FSH.

slight if any effect on urinary estrogen output. However, the combination of human pituitary FSH+HCG resulted in a marked increase of both estrogen and pregnanediol excretion.

The endometrium exhibited a secretory reaction with an almost decidua-like histologic picture. The culdoscope revealed enlarged polycystic ovaries and a fresh ovulation in one of them. A week later menstruation occurred, at which time the ovaries had resumed their normal size.

Case 5

As shown in Figure 6, the administration of HCG was followed by a marked rise in urinary pregnanediol excretion without any concomitant increase in estrogen output. The endometrial reaction was secretory and a fresh ovulation was found.

On the other hand, human pituitary FSH by itself induced a marked

rise in estrogen excretion without any appreciable increase in pregnanediol output. The proliferative activity of the endometrium was only slightly increased without any signs of a secretory reaction. Culdoscopy revealed polycystic enlarged ovaries but no fresh ovulation.

Case 6

The findings in this patient (Fig. 7) resembled those in Case 5. Following administration of HCG there was a definite rise in urinary pregnanediol excretion but only a slight increase in estrogen output. The proliferative endometrium was transformed into a secretory one.

The administration of human pituitary FSH by itself resulted in a pronounced rise in the excretion of urinary estrogen and in a moderate rise in the excretion of pregnanediol. The endometrium did not show any signs of secretory reaction.

Case 7

This adipose, hirsute woman had amenorrhea and a moderate cystic enlargement of the right ovary—Stein-Leventhal syndrome. As shown in Figure 8, she was treated with human pituitary FSH for eight days, and then by combined FSH+HCG therapy for two days.

Following the hormonal treatment, the endometrium showed a secretory reaction. Urinary excretion of pregnanediol, but not of estrogen, increased.

The day following the last injection, the patient was operated upon, and both ovaries were found to be polycystic and enlarged. In the left ovary a fresh ovulation was seen. This is shown in Figure 9.

On histologic examination, the corpus luteum was estimated to be approximately six days old. Wedge resection was performed on both ovaries. Four days after operation menstrual bleeding occurred. Four weeks later there was another episode of menstrual bleeding, and during the next period the patient became pregnant.

Summary of response of patients to different hormonal treatments

The responses of the 7 patients to the 3 types of treatment are summarized in Table 3.

In 4 cases HCG by itself did not induce ovulation and had no effect on the endometrium or on the urinary excretion of pregnanediol or of estrogen. In these patients repeated endometrial biopsies carried out before the start of treatment indicated an inactive endometrium or only slight proliferative activity (1 case). On the other hand, in 2 patients showing endometrial proliferation, the administration of HCG was followed by ovulation and a secretory transformation of the endometrium. These changes were accompanied by a marked rise in urinary pregnanediol excretion, whereas

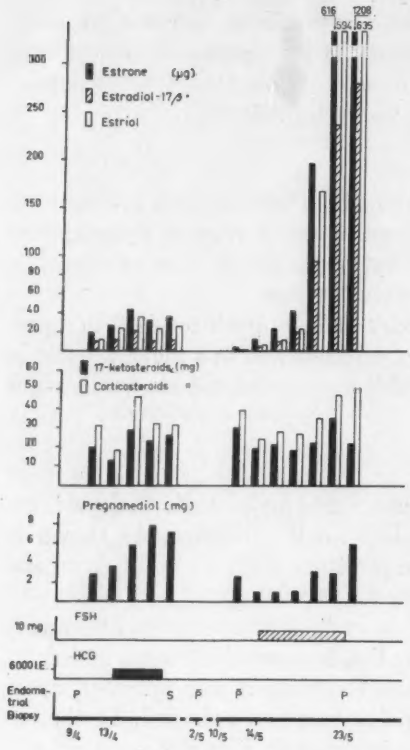

FIG. 7. Steroid excretion (48 hours) and endometrial activity in Case 6 during treatment with HCG and h.pit. FSH.

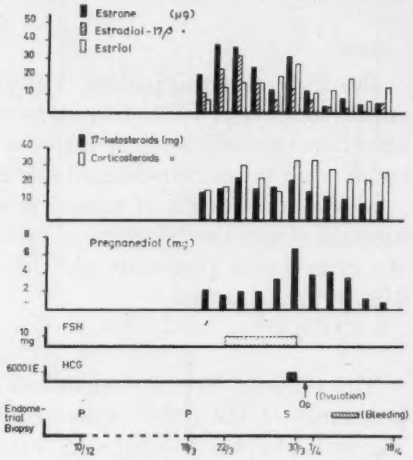

FIG. 8. Steroid excretion (48 hours) and endometrial activity in Case 7 during treatment with h.pit. FSH and HCG.

TABLE 3. RESPONSE TO HUMAN CHORIONIC GONADOTROPIN (HCG), TO HUMAN PITUITARY FSH, AND TO FSH+HCG IN 7 AMENORRHEIC WOMEN

Case No.	HCG				FSH				FSH+HCG			
	Ovulation	Endomet. response*	Urinary estrogen	Urinary pregnanediol	Ovulation	Endomet. response*	Urinary estrogen	Urinary pregnanediol	Ovulation	Endomet. response*	Urinary estrogen	Urinary pregnanediol
1	0	0	0	0					+	S	+	+
2	0	0	0	0					0	P	+	0
3	0	0	0	0					+	0	+	+
4	0	P	0	0					+	S	+	+
5	+	S	0	+	0	P	+	0				
6	+	S	0	+	0	P	+	0				
7									+	S	0	+

* P = proliferative activity.
S = secretory reaction.

estrogen excretion was practically unchanged. The administration of human pituitary FSH produced in 2 patients a pronounced rise in urinary estrogen excretion. This was accompanied by a proliferative reaction of the endometrium. Pregnanediol excretion was virtually unchanged. When human pituitary FSH therapy was combined with or followed by HCG therapy, the result was in all cases a polycystic enlargement of the ovaries. Ovulation took place in 4 out of 5 patients, and a secretory reaction of the endometrium occurred in 3 of them. These changes were paralleled by a rise in urinary pregnanediol and estrogen excretion, to a level much above that normally found during the menstrual cycle.

FIG. 9. Enlarged, polycystic ovaries with a corpus luteum in the right one, found at laparatomy in Case 7.

There were, however, a few results which require some comments. Thus, in Case 2 there was no ovulation and consequently no rise in pregnanediol excretion. Even the rise in estrogen output was rather limited when compared to that seen in the other cases. The reason for this is not well understood; it is possible that this patient might have required a higher dosage and/or a repeated course of human pituitary FSH+HCG. It also appears (Table 3) that in Case 3 there was no endometrial response, although both pregnanediol and estrogen excretion were greatly elevated. It is possible that in this case the endometrium was damaged and could not respond adequately. In Case 7 there was no increase in urinary estrogen excretion following treatment with human pituitary FSH+HCG, although ovulation took place. However, in this instance ovulation most probably occurred early in the course of FSH administration (as shown by the histologic ap-

pearance of the corpus luteum). Whether in such a patient a previous ovulation might interfere with the ability of the ovaries to respond to gonadotropic stimulation, remains to be established.

DISCUSSION

Whereas it is well established that at least 2 gonadotropic hormones, FSH and LH, can be separated from animal pituitaries, the existence of these 2 hormones as separate substances in the human pituitary is still under debate. It has been suggested by several workers that in the human subject there may be only 1 gonadotropin molecule exhibiting both FSH- and LH-activity (25–27). This suggestion was based on the fact that, so far, attempts to separate these 2 activities from human pituitaries (27) or from human urine (26, 27) by electrophoresis or by chemical fractionation (28) have not been successful. Under such circumstances it may be questioned whether it is justifiable to designate the partially purified pituitary gonadotropin used in this study as human pituitary FSH. However, when compared with human menopausal gonadotropin (HMG-20), the pituitary preparation exhibited much less LH-activity than FSH-activity. Furthermore, the fraction precipitating at an ammonium sulphate concentration of 55 per cent was found to possess LH-activity, whereas no FSH-activity could be demonstrated in this fraction at the dose levels studied. Although this evidence does not permit a final answer to the problem of duality of these hormones in the human, it suggests at least that the possibility can not be excluded that FSH and LH exist as separate substances in the human pituitary.

When discussing the clinical effects of hormonal therapy it should be recognized that it is extremely difficult, if not impossible, to differentiate between spontaneous occurrences and induced effects when observations are based on a small number of patients. It should also be remembered that our studies were not conducted on hypophysectomized subjects and that the hormone preparation was only partially purified. With these limitations in mind, a few tentative generalizations can be made.

It is apparent from the present study that the human pituitary FSH used is a highly potent hormone when administered to human beings. In amenorrheic women it produced enlarged polycystic ovaries and a great increase in the secretion of estrogen. When FSH was combined with HCG, in most cases ovulation took place and a corpus luteum was formed. This corpus luteum was capable of secreting substantial amounts of progesterone, as shown by the secretory change of the endometrium and by the greatly elevated output of urinary pregnanediol.

The present study seems also to show that in the human being, stimula-

tion of the follicular apparatus by FSH is essential for the effect of an ovulation-inducing factor. FSH by itself did not induce ovulation in 2 cases. HCG by itself did not produce ovulation in 4 cases. However, when FSH was combined with HCG, in 3 of these patients ovulation occurred.

The ovulation which took place in 2 patients during the course of HCG therapy requires further comments. Since these patients had a previous history of amenorrhea of five to six years' duration, it is tempting to believe that the ovulation was induced by HCG, and that it was not a spontaneous one. These 2 women differed from the 4 HCG-irresponsive patients, since they exhibited a distinct endometrial proliferation and a higher pre-treatment estrogen excretion, indicating a higher degree of ovarian activity. Thus, it may be assumed that the response to HCG might be conditioned by the functional status of the ovaries.

The polycystic condition of the ovaries following the administration of human pituitary FSH indicates that a large number of follicles were stimulated. This suggests that the dose administered was most probably higher than the normal requirement. This view is also supported by the very high urinary estrogen excretion values; such values do not occur during the normal menstrual cycle. In this connection it is of great interest that, although a large number of follicles were stimulated, only 1 follicle was brought to ovulation. The exact hormonal mechanism underlying this phenomenon remains to be elucidated. Also, the marked increase in urinary corticosteroid excretion in 2 patients (Cases 1 and 3) which followed combined treatment with human pituitary FSH+HCG, is not well understood. These 2 patients received higher dosages of human pituitary FSH than did the others. It remains to be established whether the increased corticosteroid excretion was due to 1) a direct stimulation of the adrenal cortex by the human pituitary FSH, 2) contamination by ACTH, or 3) an increase in metabolites (*e.g.*, pregnanetriol) of ovarian precursors, such as 17-α-hydroxyprogesterone. An increase in urinary 17-ketosteroids in 3 out of 16 patients with Stein-Leventhal syndrome, following treatment with hog or sheep pituitary FSH, has been reported by Keettel *et al.* (29). They also wondered whether the FSH caused stimulation of the adrenal cortex.

Whereas in this study a polycystic enlargement of the ovaries following treatment with human pituitary FSH was found in all patients, Keettel *et al.* (29) using hog or sheep pituitary FSH were unable to show any ovarian response in 35 normal women. In 12 out of 13 patients with Stein-Leventhal syndrome and in 1 normal woman, however, the animal preparations caused a polycystic enlargement of the ovaries. The discrepancy between the results of these 2 studies may be explained by species differences of the preparations used or by differences in dosage.

Follicle-stimulating hormone prepared from sheep pituitaries has been shown to produce ovulation in the rhesus monkey (30); however, it was found difficult to induce repeated ovulation by the use of this preparation. On the other hand, van Wagenen and Simpson (31) were successful in inducing repeated ovulation in this species by the use of a follicle-stimulating hormone preparation obtained from rhesus pituitaries. They interpreted the favorable effect as probably due to lack of antihormone formation following the repeated administration of species-specific protein hormone fraction. The findings of these workers suggest that human pituitary FSH may also be capable of producing repeated ovulation in the human. However, in the present study the effect of human pituitary FSH was investigated only in short-term studies. Except for Patient No. 7, who was also treated by wedge resection, none of the patients had a spontaneous second menstrual period. Thus it appears likely that in order to achieve a favorable therapeutic result, further series of injections will be necessary. Whether or not repeated injections will be as effective as the first one is not known. Lack of antihormone formation to this preparation remains to be established. Last, but not least, the question whether ova produced by such means can be fertilized also awaits an answer.

Acknowledgment

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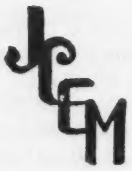
REFERENCES

1. Li, C. H.; Simpson, M. E., and Evans, H. M.: Isolation of pituitary follicle stimulating hormone (FSH), *Science* **109**: 445, 1949.
2. Steelman, S. L.; Lamont, W. A., and Baltes, B. J.: Preparation of highly active follicle stimulating hormone from swine pituitary glands, *Acta endocrinol.* **22**: 186, 1956.
3. Evans, H. M., and Simpson, M. E.: Physiology of the gonadotrophins, *in* The Hormones, ed. by G. Pincus and R. Thimann. New York, Academic Press Inc., 1950, vol. 2, p. 351.
4. Bahn, R. C.; Lorenz, N.; Bennett, W. A., and Albert, A.: Gonadotropins of the pituitary gland and the urine of the adult human female, *Endocrinology* **52**: 135, 1953.
5. Jungck, E. C., and Brown, W. E.: Human pituitary gonadotropin for clinical use: preparation and lack of antihormone formation, *Fertil. & Steril.* **3**: 224, 1952.
6. Loraine, J. A.: Clinical Application of Hormone Assay. Edinburgh, E. & S. Livingstone Ltd., 1958.
7. Diczfalusy, E., and Loraine, J. A.: Sources of error in clinical bioassays of serum chorionic gonadotropin, *J. Clin. Endocrinol. & Metab.* **15**: 424, 1955.

8. ALBERT, A.: Human urinary gonadotrophin, *Recent Progr. Hormone Res.* **12**: 227, 1956.
9. STEELMAN, S. L., and POHLEY, F. M.: Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotropin, *Endocrinology* **53**: 604, 1953.
10. GREEP, R. O.; VAN DYKE, H. B., and CHOW, B. F.: Gonadotropins of the swine pituitary. I. Various biological effects of purified thylakentrin (FSH) and pure metakentrin (ICSH), *Endocrinology* **30**: 635, 1942.
11. LORAINE, J. A., and BROWN, J. B.: Some observations on the estimation of gonadotrophins in human urine, *Acta endocrinol.* **17**: 250, 1954.
12. DICZFALUSY, E.: An improved method for the bioassay of chorionic gonadotrophin, *Acta endocrinol.* **17**: 58, 1954.
13. BORTH, R.; DICZFALUSY, E., and HEINRICHS, H. D.: Grundlagen der statistischen Auswertung biologischer Bestimmungen, *Arch. f. Gynäk.* **188**: 497, 1957.
14. GADDUM, J. H.: Polypeptides which Stimulate Plain Muscle. Edinburgh, E. & S. Livingstone Ltd., 1955.
15. EVANS, H. M.; SIMPSON, M. E.; MARX, W., and KIBRICK, E.: Bioassay of the pituitary growth hormone. Width of the proximal epiphysial cartilage of the tibia in hypophysectomized rats, *Endocrinology* **32**: 13, 1943.
16. BROWN, J. B.: A chemical method for the determination of oestriol, oestrone and oestradiol in human urine, *Biochem. J.* **60**: 185, 1955.
17. DICZFALUSY, E., and WESTMAN, A.: Urinary excretion of natural oestrogens in oophorectomized women treated with polyoestradiolphosphate (P.E.P.), *Acta endocrinol.* **21**: 321, 1956.
18. BROWN, J. B.; BULBROOK, R. D., and GREENWOOD, F. C.: An additional purification step for a method for estimating oestriol, oestrone and oestradiol-17β in human urine, *J. Endocrinol.* (British) **16**: 49, 1957.
19. KLOPPER, A.; MICHIE, E. A., and BROWN, J. B.: A method for the determination of urinary pregnanediol, *J. Endocrinol.* (British) **12**: 209, 1955.
20. ALLEN, W. M.: A simple method for analyzing complicated absorption curves, of use in the colorimetric determination of urinary steroids, *J. Clin. Endocrinol.* **10**: 71, 1950.
21. DICZFALUSY, E.: Experimental verification of the assumption underlying the colour correction equation of Allen, *Acta endocrinol.* **20**: 216, 1955.
22. VESTERGAARD, P.: Rapid micro-modification of the Zimmermann/Callow procedure for the determination of 17-ketosteroids in urine, *Acta endocrinol.* **8**: 193, 1951.
23. APPLEBY, J. I.; GIBSON, G.; NORYMBERSKI, J. K., and STUBBS, R. D.: Indirect analysis of corticosteroids. I. The determination of 17-hydroxysteroids, *Biochem. J.* **60**: 435, 1955.
24. BIRKE, G.; DICZFALUSY, E., and PLANTIN, L.-O.: Assessment of the functional capacity of the adrenal cortex. I. Establishment of normal values, *J. Clin. Endocrinol. & Metab.* **18**: 736, 1958.
25. SEGALOFF, A.: *In* Year Book of Endocrinology, ed. by G. S. Gordan. Chicago, Year Book Publishers, Inc., p. 266, 1954–55.
26. STRAN, H. M., and JONES, G. E. S.: Some properties of human urinary gonadotrophins as elaborated by filter paper electrophoresis, *Bull. Johns Hopkins Hosp.* **95**: 162, 1954.
27. RIGAS, D. A.; PAULSEN, C. A., and HELLER, C. G.: Purification of gonadotrophins derived from urine and pituitary glands of human beings: observations on their electrophoretic behavior and biological activity, *Endocrinology* **62**: 738, 1958.

28. JOHNSEN, S. G.: Studies on the urinary gonadotrophins. II. Further purification of highly active gonadotrophins from the urine of postmenopausal women, *Acta endocrinol.* **20**: 106, 1955.
29. KEETTEL, W. C.; BRADBURY, J. T., and STODDARD, F. J.: Observations on the polycystic ovary syndrome, *Am. J. Obst. & Gynec.* **73**: 954, 1957.
30. HISAW, F. L.: Development of the Graafian follicle and ovulation, *Physiol. Rev.* **27**: 95, 1947.
31. VAN WAGENEN, G., and SIMPSON, M. E.: Induction of multiple ovulation in the rhesus monkey (*Macaca mulatta*), *Endocrinology* **61**: 316, 1957.



FAMILIAL TRUE HERMAPHRODISM IN PRE- AND POSTPUBERAL GENETIC FEMALES. HORMONAL AND MORPHOLOGIC STUDIES

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ABSTRACT

The clinical findings, urinary hormonal patterns and gonadal morphology in 2 genetic female siblings with scrotal testes and ovaries are presented. As far as is known, this is the first reported instance of familiar true hermaphrodism. The testes of the postpuberal sibling showed seminiferous tubule dysgenesis which was identical to that found in Klinefelter's syndrome, whereas the testes of the prepuberal sibling were histologically normal. These findings are particularly significant in the natural history of seminiferous tubule dysgenesis.

A CASE of true hermaphrodism (1) was recently reported in which there were histologically well developed and separate testes and ovaries found bilaterally in the scrotum. It was suggested that this represented a marked form of sex reversal but to a degree less than that found in the chromatin-positive Klinefelter "male." We have recently seen 2 siblings with identical defects in gonadal development which were felt to be similar to those in the case reported earlier. The purpose of this communication is to describe the clinical findings, hormonal patterns, and gonadal morphology of these siblings, one of whom was prepuberal and the other postpuberal.

CASE HISTORIES AND METHODS

D.R.B. (Fig. 1), aged 16, was referred to the endocrine service because of progressive enlargement of the breasts beginning at about age 13. Except for a congenital abnormality of the penis described as "complete hypospadias without cryptorchidism" which had been successfully repaired at age 4, the patient had experienced excellent general health and development, and was a psychologically well orientated male. Concomitant with the normal puberal changes—growth spurt, increase in size of the penis, appearance of abundant sexual hair, deepening of the voice, and development of comedones and acne—there had been progressive asymptomatic symmetrical enlargement of the breasts. In retrospect the testes were noted to have remained small and insensitive.

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FIG. 1. Patient D.R.B., aged 16 years. This photograph was taken subsequent to scrotal exploration; hence the absence of pubic hair.

FIG. 2. Patient R.F.B., aged 7 years.

The family history was significant, in that a 7-year-old brother (R.F.B.) had a similar congenital abnormality of the external genitalia. A 12-year-old brother was normal and there were no other familial instances of abnormal sexual development.

Physical examination revealed an apparently well adjusted, healthy 16-year-old white male who was normal except for a moderately tall (5 feet $8\frac{1}{2}$ inches) eunuchoid body build and abnormal sexual development. The breasts (subsequently removed) were symmetrically enlarged with firm glandular tissue which measured 9 cm. in diameter. The areolae and nipples were pigmented and well developed.

The penis, which measured 9 cm. $\times 2\frac{1}{2}$ cm., was straight, capable of erection and normal in appearance except for the position of the urethal meatus, which was 1.5 cm. from the tip of the corona. The scrotum was well developed, pigmented and wrinkled. The right gonad measured 2.5 cm. $\times$ 1.7 cm. and was very firm, irregular, and insensitive. There was a smaller, soft, vaguely defined mass adjacent inferiorly to the gonad, which had the consistency of an epididymis. The left gonad, which measured 2.7 cm. $\times$ 1.6 cm., was also insensitive but less irregular. An epididymis-like mass was also palpable beneath this gonad. The prostate was normally enlarged to young adult size. There was a normal male escutcheon of pubic hair. Axillary hair was abundant and a beard was beginning on the upper lip. There was excessively long hair on the legs anteriorly just below the patellae, but in no other areas. The voice was moderately deep. There were

numerous comedones and acneform lesions on the back, and a smaller number on the anterior chest and face.

A smear of the buccal mucosa demonstrated a positive nuclear chromatin pattern, but urethroscopy and urethrograms failed to reveal a urogenital sinus or other vestiges of the Müllerian system. Hormonal studies (Table 1) showed a low urinary excretion of 17-ketosteroids, an elevated excretion of estrogens and pregnanediol, and a normal excretion of gonadotropins.

The initial impression was Klinefelter's syndrome; however, the occurrence of familial hypospadias suggested that a more complex type of gonadal dysgenesis existed.

R.F.B. (Fig. 2), the 7-year old sibling, was born prematurely (at 7 months). The

TABLE 1. PREOPERATIVE AND POSTOPERATIVE EXCRETION OF HORMONES IN THE URINE OF THE SIBLINGS

Patient	Time	17-KS (mg./24 hrs.)	Pregnanediol (mg./24 hrs.)	Estradiol-17β (μg./24 hrs.)	Estrone (μg./24 hrs.)	Gonadotropin (m.u./24 hrs.)
			Before operation			
		2.8	5.3	27.6	30.8	16–50
		4.3	5.9	23.3	38.0	
D.R.B., aged 16			*After right oophorectomy*			
	2½ wks.	4.5	3.6	18.7	13.4	16–50
	5 mo.	2.8	0.33	16.5	21.6	
	7½ mo.	3.7				
			Before operation			
		1.2	0.4	5.7	9.9	less than 6
R.L.B., aged 7			*After bilateral oophorectomy*			
	5 mo.	1.9	0.0	6.5	12.2	
	9½ mo.	1.4				

birth weight was 3 pounds 6 ounces, and the infant at that time was considered normal except for the presence of a defect in the external genitalia. The defect, which was identical to that of D.R.B., consisted of a phallus of normal size which was bound down ventrally and lacked a urethral meatus. Instead, urine was emitted through a small opening at the base of the phallus. The normal-appearing scrotum contained palpable gonads bilaterally. An abdominal exploration which was carried out in infancy revealed no female internal genitalia. The construction of a functional penile urethra was completed at the age of 6 years. The child had had good general health with normal growth and development.

Physical examination revealed a healthy 7-year-old white boy of normal height and weight with the significant physical abnormalities limited to the genitalia. The penis, which measured 4.5 cm. in length, was slightly hooded at the base with a fold of skin, but otherwise appeared normal except for old operative scars on the ventral surface. The urethral meatus was centrally placed in the corona. The scrotum was well developed and contained very mobile gonads which had a normal consistency despite their small

size. The right gonad measured 1.6 cm.×1.1 cm. and the left, 1.8 cm.×1.2 cm. There was no evidence of sexual maturation.

In this child also there was a positive nuclear chromatin pattern in the buccal smears and a urethrogram showed that there were no Müllerian vestiges. Studies of 24-hour urine samples (Table 1) revealed a normal excretion of 17-ketosteroids and estrogens, a slightly elevated excretion of pregnanediol, and a gonadotropin excretion of less than 6 mouse units.

Urinary 17-ketosteroids were measured by the Zimmermann reaction, according to a modification of the method of Callow *et al.* (2). The 24-hour urinary excretion of 17-ketosteroids for prepuberal males in this laboratory ranges between 1 and 3 mg., and that for postpuberal males between 7 and 12 mg.

Urinary pregnanediol excretion was determined by a slight modification of the method of Eberlein and Bongiovanni (3). Prior to chromatography on washed filter paper, the Girard procedure as described by Schneider (4) was carried out on the residues. Sulfuric-acid chromogens of the filter-paper eluates and authentic pregnanediol were used in the calculations. The range of urinary pregnanediol excretion for normal adult females is 0.4 to 4.5 mg. per twenty-four hours.

Urinary gonadotropin assays were performed by BioScience Láboratories, Los Angeles, California.

Determinations of urinary estradiol-17β excretion were carried out by incubating aliquots of urine with β-glucuronidase and comparing the fluorescence of the residue of

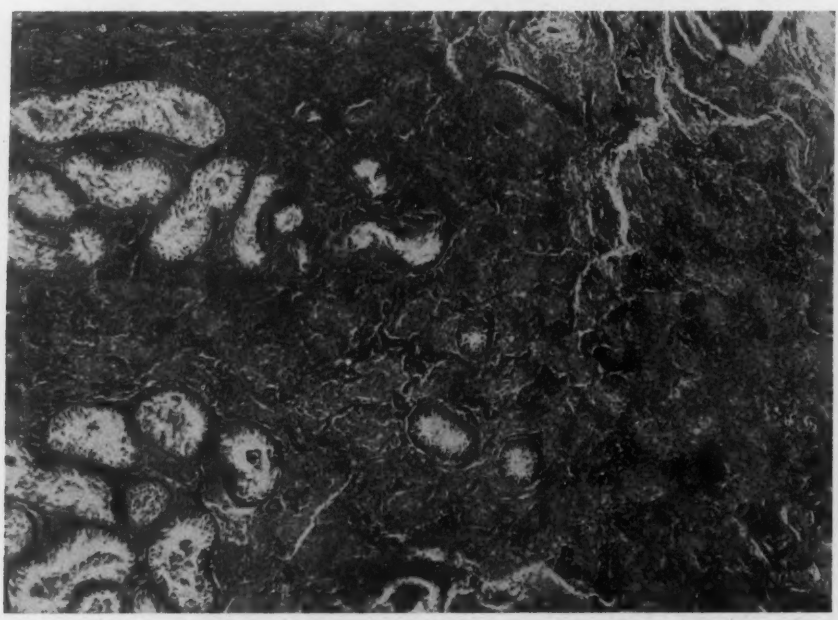

FIG. 3. Section of testis from Patient D.R.B. showing large areas of dense bands of hyalinized material representing collapsed tubules. A few of the tubules are of the small, immature type, with thickened lamina propria. (Weigert-van Gieson ×40.)

the urine after benzene extraction and purification with that of authentic crystalline standard in 30 *N* sulfuric acid. Estrone excretion was similarly determined on the same aliquot of urine after hydrolysis by boiling with acid. Using this method, the 24-hour excretion of estradiol-17β or estrone for adult females is approximately 60 μg. per twenty-four hours.

Gonadal Morphology

D.R.B. Exploration of the scrotal contents revealed firm, atrophic testes, each measuring approximately 2.5 cm. $\times$ 1.5 cm. At the upper pole of the right testis there was a firm, whitish mass, 1 cm. in diameter. This mass was removed at the time biopsy specimens were taken from the right testis and epididymis. The corresponding mass was not identified when the biopsy specimen was taken from the left testis.

Microscopically (Fig. 3), the testes were made up of architecturally distorted and numerically decreased seminiferous tubules embedded in dense bands of hyalinized material. This hyalinized material consisted predominantly of collagen and represented collapsed seminiferous tubules from which all cellular elements had disappeared. Two types of tubules were present: 1) those of small caliber lined by an epithelium made up of one or two layers of undifferentiated cells, with thickening of the lamina propria being the only evidence of degeneration, and 2) large, dilated tubules with thickened sclerotic walls lined by sparsely cellular epithelium consisting primarily of Sertoli cells but also containing a few spermatogonia. The interstitial tissues were filled with large clumps of round or elongated Leydig cells (Fig. 4).

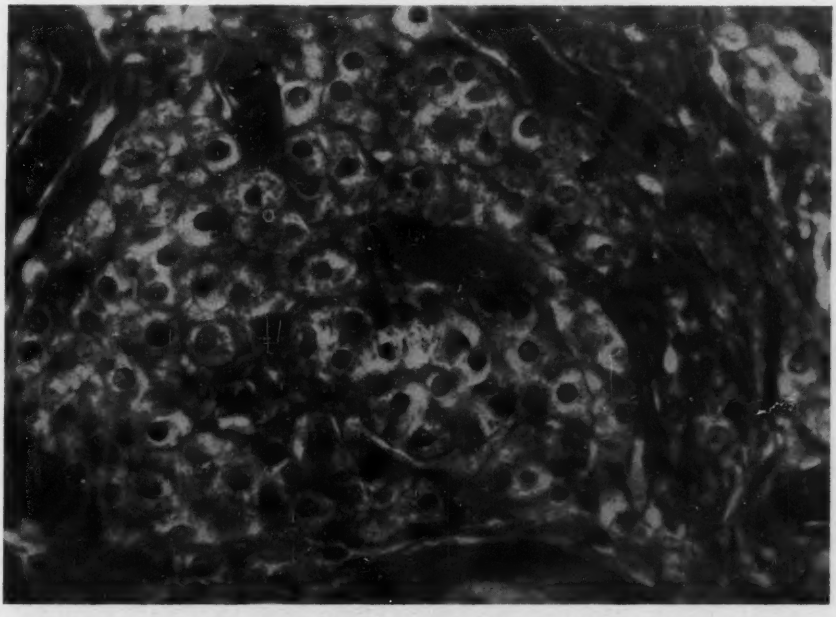

FIG. 4. Section of testis from Patient D.R.B. showing a large clump of Leydig cells. (Mallory's aniline blue $\times$450.)

Sections of the 1-cm. oval mass from the upper pole of the right testis revealed ovarian tissue (Fig. 5) consisting almost entirely of stromal elements in which a few scattered primordial and atretic follicles were identified.

In the sections of the normal-appearing epididymis, there was an additional tubular structure embedded within an irregular sheet of smooth muscle, which was lined by a pseudostratified columnar ciliated epithelium with a few secretory cells. This structure was presumably a vestigial Fallopian tube.

Scrotal exploration in Patient R.F.B. (Fig. 6) revealed testes measuring 1.6 cm. by 1.1 cm., which were normal in appearance. At the upper pole of each testis was a small, firm, whitish, oval mass approximately 0.6 cm. in length. Each oval structure appeared to be separate from the testis, but incorporated in a fibrous sheath with it. In addition to the normal-appearing epididymis on each side, there were tubular masses which (although no biopsies were performed) were felt to be rudimentary Fallopian tubes. The small oval masses were removed and biopsy specimens were taken from the testes.

Microscopic examination of the testes (Fig. 7) revealed compact, well formed, cellular seminiferous tubules with walls of normal thickness. Many tubules had ill-defined lumens filled with a finely granular eosinophilic material. The lining cells were large and undifferentiated and there were no Leydig cells in the interstitial spaces. Sitting cap-like on each testis, which was surrounded by a thin fibrous capsule, was a section of well-formed ovary (Fig. 8) which was also surrounded by a fibrous capsule. Within the ovary, there was abundant stroma with numerous peripherally placed primordial follicles. A few partially maturing follicular cysts were identified. Connecting the ovary with the testis (Fig. 9) was a small area of tissue in which unicellular tubules merged with seminiferous tubules suggestive of rete.

It is of interest that peripheral clumping of nuclear chromatin, characteristic of the female, was demonstrable in many Leydig cells in the testes of Patient D.R.B. and in the seminiferous tubule cells of Patient R.F.B.

DISCUSSION

The finding in these genetic female siblings suggested that, although the cortical component of the indifferent bipotential gonad had undergone development, there was either failure of normal suppression of the medullary element or a stimulus to its development resulting in the formation of both testes and ovaries. It would appear that failure of migration of the primordial germ cells to the genital ridge—postulated as a cause of persistence and development of the medullary component in genetic females—was not a factor in these cases. That the defect was of genetic origin was indicated by the identical gonadal differentiation of the siblings. As far as is known, this is the first report of hermaphrodism of this type. Outstanding reviews of the concepts of the role of genetic and gonadal factors in disorders of gonadogenesis have recently been published by Grumbach *et al.* (5) and Witschi *et al.* (6).

The finding of hermaphroditic genitalia in these patients, as well as in the previously reported 8-month-old infant (1), would suggest that the fetal ovary may have had an active but inadequate role in genital development.

Seminiferous tubule destruction, which in the older sibling was identical

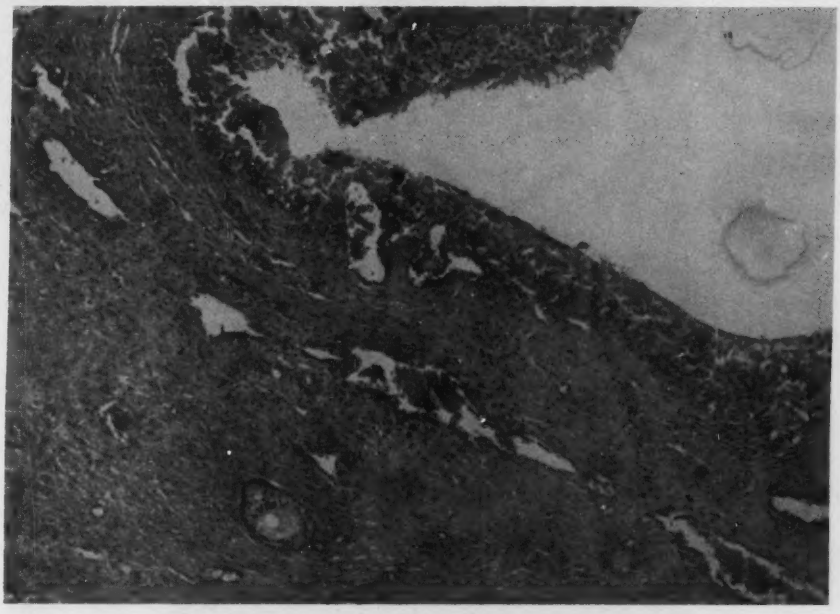

FIG. 5. A maturing and an atretic follicle in the ovarian tissue of Patient D.R.B. (Hematoxylin-eosin ×40.)

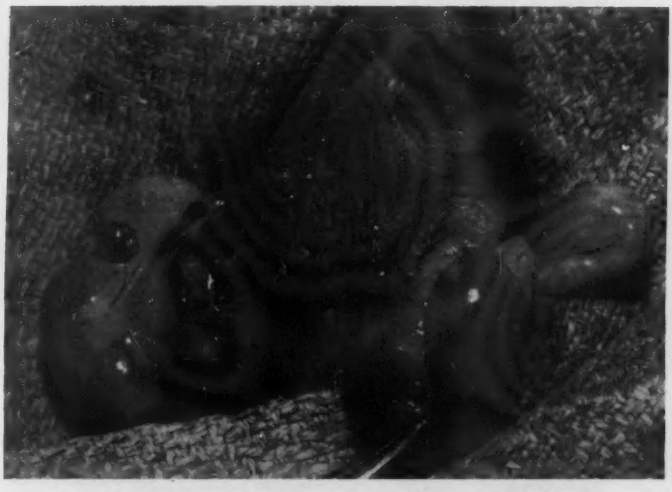

FIG. 6. Scrotal contents—Patient R.F.B. Separate testis and ovary can be seen distinctly on the left-hand side.

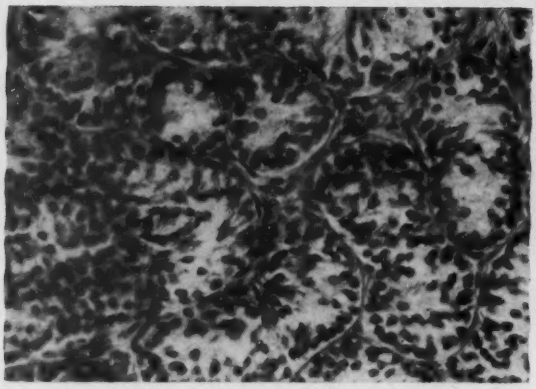

FIG. 7. Section of normal-appearing testis from Patient R.F.B.
(Hematoxylin-eosin ×200.)

FIG. 8. Section of normal-appearing ovary from Patient R.F.B.
(Hematoxylin-eosin ×25.)

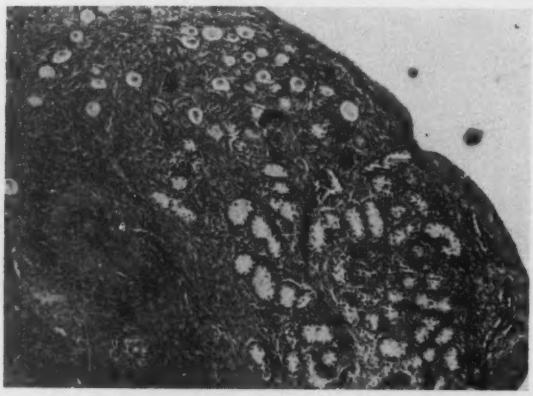

FIG. 9. Section from the junction of ovary and testis in Patient R.F.B.
(Hematoxylin-eosin ×25.)

to that found in chromatin-positive Klinefelter "males," undoubtedly began at puberty, since the prepuberal sibling as well as the 8-month-old infant with an identical defect had testes that appeared entirely normal in architecture. It is possible that because of an inherent genetic defect, these testes which were functionally predominant *in utero* and appeared normal prepuberally, were incapable of maturing and degenerated when stimulated with follicle-stimulating hormone. However, the presence of functional ovarian tissue in the case of the older sibling cannot be discounted as an influence on the initiation and progression of tubule destruction. Of considerable interest will be 1) the postpuberal testicular morphology of the younger patients with this type of true hermaphrodism following oophorectomy, and 2) whether or not gynecomastia will occur, as in some cases of Klinefelter's syndrome.

The absence of elevated gonadotropin excretion in the older sibling was thought to be due to the suppressive effect of estrogens. The urinary estrogen levels found after removal of the right ovary were undoubtedly due to left-sided ovarian tissue which was not identified at exploration. Although the urinary pregnanediol levels were considerably lower after surgery, this was probably due to the cyclic secretion of progesterone rather than to removal of all of the ovarian tissue. The urinary level of pregnanediol found in the younger sibling remains unexplained.

A family has been reported in which 9 out of 10 of the male members of 2 generations had Klinefelter's syndrome (6). Although some of these had hypospadias, the report is vague as to the gonadal morphology. It is suggested that in cases of Klinefelter's syndrome with genital defects, a thorough exploration of the scrotal contents is indicated.

Acknowledgment

Doctors Harry Grabstald and Michael K. O'Heeron performed the scrotal explorations and oophorectomies. We gratefully acknowledge the help given by Miss Carol Carlisle in carrying out the hormone assays.

Addendum

Since submitting this communication, an article has appeared reporting 2 siblings with true hermaphrodism in whom the chromatin pattern was negative (Milner, W. A.; Garlick, W. B.; Fink, A. J., and Stein, A. A.: *J. Urol.* **79**: 1003, 1958).

REFERENCES

1. Clayton, G. W.; O'Heeron, M. K.; Smith, J. D., and Grabstald, H.: A case of true hermaphrodism: possible relationship to Klinefelter's syndrome, *J. Clin. Endocrinol. & Metab.* **17**: 1002, 1957.
2. Callow, N. H.; Callow, R. K., and Emmens, C. W.: Colorimetric determination of substances containing the grouping—$CH_2 \cdot CO$—in urine extracts as an indication of androgen content, *Biochem. J.* **32**: 1312, 1938.

3. EBERLEIN, W. R., and BONGIOVANNI, A. M.: A paper chromatographic method for measurement of pregnanediol in urine, *J. Clin. Endocrinol. & Metab.* **18**: 300, 1958.
4. SCHNEIDER, J. J.: Further isolation of adrenocortical compounds from male urine, *J. Biol. Chem.* **194**: 337, 1952.
5. GRUMBACH, M. M.; BLANC, W. A., and ENGLE, E. T.: Sex chromatin pattern in seminiferous tubule dysgenesis and other testicular disorders: relationship to true hermaphrodism and Klinefelter's syndrome, with a review of gonadal ontogenesis, *J. Clin. Endocrinol. & Metab.* **17**: 703, 1957.
6. WITSCHI, E.; NELSON, W. O., and SEGAL, S. J.; Genetic, developmental and hormonal aspects of gonadal dysgenesis and sex inversion in man, *J. Clin. Endocrinol. & Metab.* **17**: 737, 1957.
7. REIFENSTEIN, E. C., JR.: Hereditary familial hypogonadism, *Proc. Am. Fed. Clin. Res.* **3**: 86, 1947.

SEMINIFEROUS TUBULE DYSGENESIS (KLINEFELTER'S SYNDROME) IN IDENTICAL TWINS*

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ABSTRACT

Seminiferous tubule dysgenesis (Klinefelter's syndrome) was proved by testicular biopsy in 17-year-old identical twins who presented with the typical clinical features of the syndrome. Female nuclear chromatin patterns were present in the Leydig cells and in buccal mucosal smears of both patients. The identity of the twins was established by means of reciprocal homografts, blood antigen studies, dermatoglyphics and the history of a single placenta.

The principal theories concerning the etiology of this syndrome are briefly reviewed. It is concluded that concordance for seminiferous tubule dysgenesis in identical twins in the light of previous evidence supports the concept that a genetic predisposition exists in this disorder.

KLINEFELTER'S syndrome or, as it has been designated more recently, seminiferous tubule dysgenesis (1, 2), has been recognized with increasing frequency since the original description in 1942 (3). This is partly attributable to a clearer definition of the syndrome. Gynecomastia, eunuchoidism and elevated urinary gonadotropins are now recognized to be frequent, rather than obligatory features of the clinical picture (4). In addition, typical histopathologic changes have been discovered in the testes of patients whose sole complaint is sterility (1, 5). Although originally thought to begin at puberty (3, 4), seminiferous tubule dysgenesis is now considered by most workers to be a congenital disorder which is manifested symptomatically only after puberty (1, 6–9).

Despite agreement concerning its congenital nature, the etiology and pathogenesis of seminiferous tubule dysgenesis are as yet unknown (2). A recent advance in our knowledge of this problem occurred with the discovery that many patients with this syndrome had nuclear chromatin patterns similar to those seen in genetic females (10–13). This apparent dis-

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† Fellow of the National Foundation for Infantile Paralysis.

crepancy between chromosomal sex and phenotypic and gonadal sex relegated seminiferous tubule dysgenesis to the general category of human intersexuality. Similar discordances between somatic and chromosomal sex have been noted previously in gonadal dysgenesis (14, 15), pseudohermaphrodism and true hermaphrodism (2, 16, 17).

A report of identical twins with seminiferous tubule dysgenesis and female nuclear chromatin patterns is presented and the significance of this occurrence is considered.

CASE HISTORIES

Case 1

A.W. (P. H. No. 1329995), a 17-year-old schoolboy, was admitted to the Presbyterial Hospital for investigation of delayed puberty and bilateral gynecomastia. At birth, the patient was one of twins; records of the birth indicate the presence of a single placenta. His growth and development were entirely normal. There were no serious illnesses or hospitalizations. Mumps and orchitis were specifically denied.

At the age of about 15 years, the patient noted that his breasts had enlarged; at the same time, it was apparent that his genitalia, body hair and beard were not undergoing the normal puberal development. Although nocturnal emissions began to occur at age 15, the patient had no interest in girls, nor had he found it necessary to shave. His family doctor treated him with "hormones" for a brief period without effect.

Physical examination showed a eunuchoid habitus, with distribution of adipose tissue about the hips and poor muscular development (Fig. 1). His voice was high-pitched and his behavior impressed several observers as effeminate. Anthropometric data are presented in Table 1. No facial hair or beard was present; there was scanty axillary hair and moderately heavy pubic hair (of feminine distribution). Gynecomastia was present bilaterally, both glandular and fatty tissue being palpable. The penis was 3.2 cm. in diameter and 7.4 cm. in length. The testes were small. The findings on physical examination were otherwise unremarkable.

Results of routine laboratory examinations were all within normal limits. Urinary excretion of 17-ketosteroids (Zimmermann method) was 5.9 mg. per twenty-four hours. The 24-hour excretion of gonadotropin was positive at 50 mouse units and equivocally positive at 80 mouse units. A smear of the oral mucosa showed 50 to 60 per cent of the cellular nuclei to be chromatin-positive (*i.e.*, female chromatin pattern). Psychiatric evaluation, including psychometric testing, revealed a low average intellectual level, associated with considerable anxiety and emotional immaturity.

The right testis measured 1.8 cm. $\times$ 1.3 cm. and the left testis, 1.0 cm. $\times$ 1.5 cm.; both were described as having a gelatinous consistency. Microscopic sections from biopsy specimens demonstrated numerous tiny dysgenetic tubules which were completely hyalinized (Fig. 2). A few large tubules were lined with vacuolated Sertoli cells. Several isolated tubules showed spermatogenic activity. One tubule contained spermatids and a few spermatozoa (2). Only minimal interstitial-cell hyperplasia of the diffuse type was seen. The nuclear pattern of the Leydig cells was female.

Subsequently, bilateral mammoplasty was performed to correct the gynecomastia. Histologic sections showed mammary ducts imbedded in a dense fibrous connective-tissue stroma; no lobules were present. The pattern was considered "typical of gynecomastia, although the fibrous stroma is unusually dense." Soon thereafter, the patient

received a full thickness skin graft from his twin brother (Case 2); this graft, as well as a control autograft, healed uneventfully in less than three weeks. A graft from this patient to his brother was similarly successful. All grafts have been observed for more than one year.

The patient recovered from the surgical procedures uneventfully. He has been followed in clinic, and treated with testosterone.

Case 2

F.W. (P. H. No. 1330235), the twin brother of A.W. (Case 1), was also admitted to Presbyterian Hospital for evaluation of his sexual development. His past medical history did not differ from that of his brother, except that he had undergone a minor surgical procedure at the age of 4 years for a "tongue-tied condition."

This patient, like his twin, showed no secondary sexual characteristics and had never manifested any interest in girls. Slight enlargement of the breasts, which had been present for two years, was his sole complaint at the time of admission.

Physical examination revealed a eunuchoid habitus (Fig. 3) without, however, the feminine distribution of adipose tissue or the effeminate manner noted in his twin. His voice was high-pitched. Anthropometric data are presented in Table 1. There was no facial hair or beard, nor was there any hair on his chest. Axillary hair was sparse but there was a moderately profuse growth of pubic hair (feminine distribution). The penis measured 3.5 cm. in diameter and 7.6 cm. in length. The testes were small. Palpation revealed gynecomastia, though this was not apparent on casual visual examination. The remainder of the physical findings were within normal limits.

Results of routine laboratory examinations were all within normal limits. In smears

TABLE 1. SEMINIFEROUS TUBULE DYSGENESIS IN 17-YEAR-OLD IDENTICAL TWINS

	Patient A.W.	Patient F.W.		Patient A.W.	Patient F.W.
Weight (lbs.)	162	147	Sex chromatin pattern	Female	Female
Height (ins.)	74½	74½	Urinary 17-ketosteroids (mg./24 hrs.)	5.9	10.1
Upper segment / Lower segment (ins.)	33 / 41½	32¼ / 42¼	Urinary gonadotropin (mouse units/24 hrs.)	>50	>50
Span (ins.)	75½	76½	Bone age	Normal	Normal
Gynecomastia	Marked	Moderate	Dermatoglyphics	Compatible with homozygosity	
Secondary sexual characteristics	Minimal	Minimal	Reciprocal homografts of skin	Accepted	Accepted
Testicular size (at time of biopsy) (cm.)	R: 1.8×1.3 L: 1.5×1.0	R: 1.8×1.5 L: 1.8×1.5	Blood group antigens	Identical	
Phallus: length / diameter (cm.)	7.4 / 3.2	7.6 / 3.5	Testicular structure	Pathognomonic for sem. tubule dysgenesis	

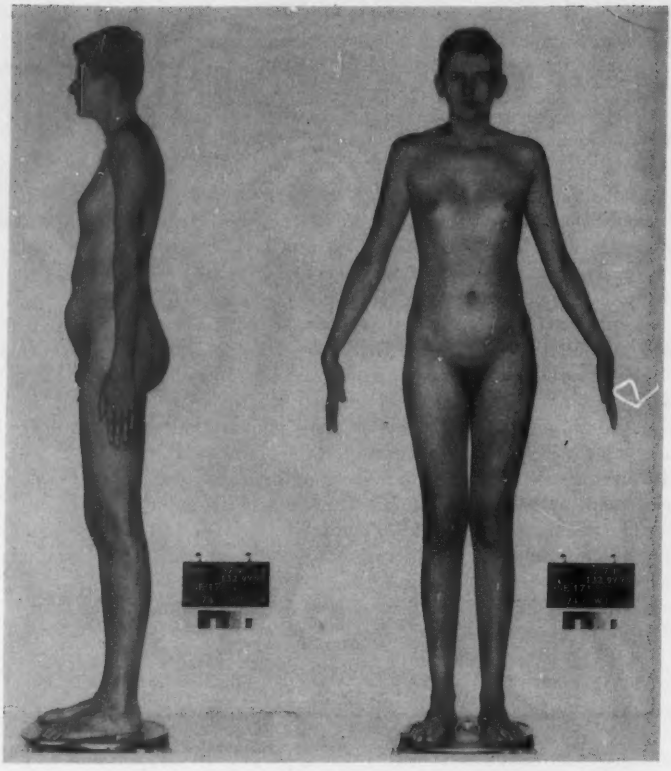

FIG. 1. Case 1 (A.W.), demonstrating eunuchoid habitus, gynecomastia, small genitalia and paucity of body hair. Pubic hair, initially present in feminine distribution, was shaved prior to testicular biopsy.

of the buccal mucosa, 50 to 60 per cent of the cells contained nuclear chromatin. Urinary gonadotropin titers were positive at 50 mouse units and equivocally positive at 80 mouse units per twenty-four hours. Urinary corticoids were 8.6 mg. per twenty-four hours (Porter-Silber method) and urinary 17-ketosteroids were 10.1 mg. per twenty-four hours. Psychologic tests showed the patient's intelligence to be in the low average range (three I.Q. points below that of his brother); his personality pattern revealed anxiety and fear associated with an awakening sexuality.

Testicular biopsy revealed both testes to measure 1.8 cm. $\times$ 1.5 cm. Microscopic sections showed the presence of small sclerotic tubules and rare, tiny, slightly sclerosed tubules lined with Sertoli cells (Fig. 4). There were numerous large tubules containing only Sertoli cells, but no spermatogenic activity was seen. Moderate interstitial-cell hyperplasia was present. The nuclear pattern of the Leydig cells was female.

Fingerprints of both twins were obtained. The 2 sets of patterns showed minor variations from each other, but the similarities were such that the fingerprints were considered compatible with monozygosity. Blood antigens were identical in the 2 patients.[1]

[1] A_1B, D+, C+, E−, c+, e+, K−, Fy^a+, JK^a+, M+, N+, P+.

Exchange skin grafts with his twin were performed successfully, as noted in Case 1. The patient has been followed in the clinic and treated with testosterone.

Family history

The parents of the twins were apparently unrelated; there was no history of consanguinity in the 2 preceding generations. Prior to the birth of the twins, the mother had been pregnant 9 times, with 7 normal deliveries and 2 spontaneous abortions. Two normal children were born subsequent to the birth of the twins.

Of 8 maternal siblings, 4 were male and all produced children. Two of the maternal siblings were dizygotic twins. Of 5 paternal siblings, 3 were male and all proved fertile. One of the twins' paternal aunts was born with deaf-mutism, the sole congenital abnormality noted on either side of the family throughout the 4 generations for which information is available. Among the numerous first cousins of the twins, there was no known instance of a syndrome similar to that described in the twins; all of the cousins of both sexes who were old enough to have married had children of their own.

The twins had 3 brothers and 6 sisters. With the exception of 2 brothers (1 of whom is married and has 3 children), all of the siblings were examined by the authors. They all exhibited sexual development and function appropriate to their respective ages, and all were free of obvious congenital abnormality. In all cases, smears from the buccal mucous membrane provided evidence for agreement between genotypic and phenotypic sex; this was also true for the parents of the twins. Similarly, serologic tests for 12 blood groups in the parents and siblings disclosed no pertinent discrepancy.

DISCUSSION

These patients are examples of seminiferous tubule dysgenesis in all of its major manifestations, *i.e.*, small testes, azoospermia, elevated urinary gonadotropins, eunuchoid habitus, poorly developed or absent secondary

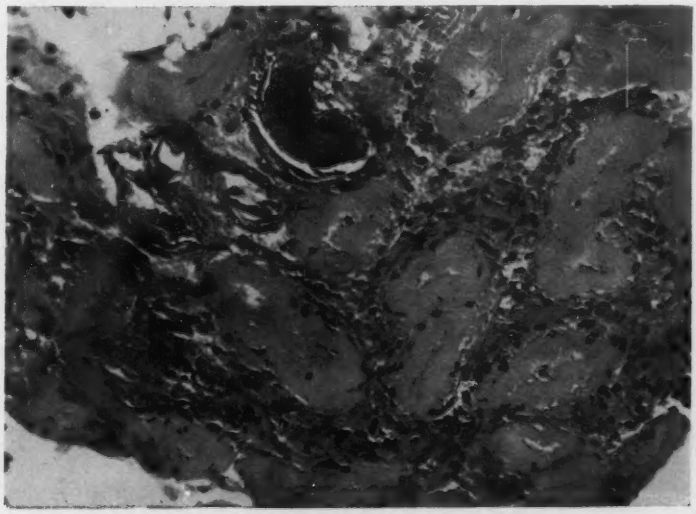

FIG. 2. Testicular tissue, Case 1, showing completely fibrosed dysgenetic tubules. (Hematoxylin-eosin ×223.)

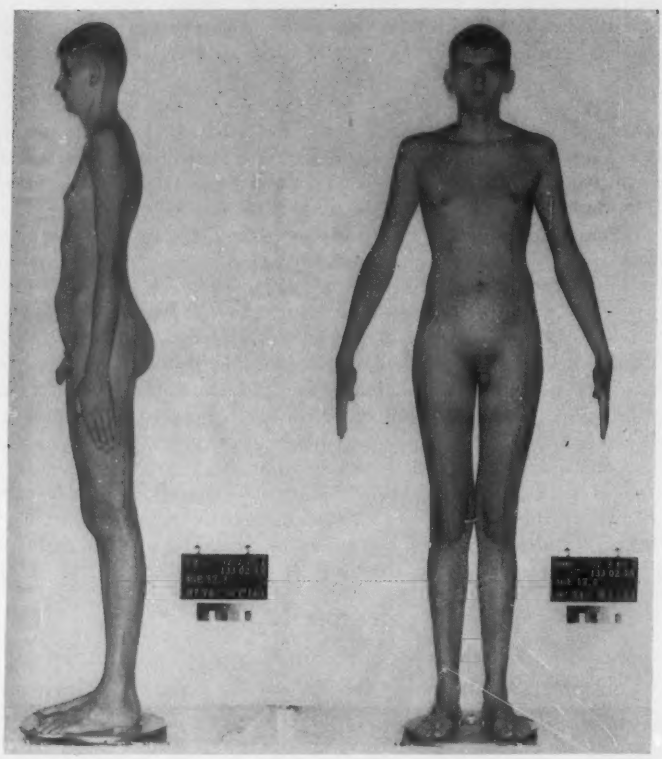

FIG. 3. Case 2 (F.W.), demonstrating eunuchoid proportions, gynecomastia and small genitalia, but a somewhat more masculine habitus than that in A.W. (Case 1). Pubic hair was shaved prior to testicular biopsy.

sex characteristics, gynecomastia, and typically sclerotic and dysgenetic seminiferous tubules. Moreover, the chromosomal sex in each case was female, firmly establishing the fact that the tubular lesions were congenital rather than the result of acquired testicular disease.

The presence of a single placenta, identity of blood antigens, physical and dermatoglyphic similarities, and acceptance of reciprocal homografts combine to establish the identity of the twins. The psychologic differences between the 2 patients were unusual but must be regarded as environmentally determined. Similarly, the minor differences in physical appearance between the twins (including the more apparent gynecomastia in A.W. than in F.W.) probably represent differences in eating habits and distribution of adiposity rather than a true discordance in somatic development.

Although these are the first reported cases of seminiferous tubule dysgenesis occurring in identical twins, the familial occurrence of the syndrome has been described by several others. Sohval and Soffer (18) reported 2 brothers, both chromosomal males (19), in whom the syndrome was associated with mental deficiency and multiple congenital osseous abnormalities. Bunge and Bradbury (6) and Grumbach, Blanc and Engle (1) have observed pairs of brothers with seminiferous tubule dysgenesis; in each pair, one of the siblings was chromatin-positive and the other chromatin-negative. Nadler *et al.* (20) observed a pair of brothers with the typical clinical picture and testicular histologic pattern, in whom dystrophia myotonica was also present. Reifenstein (21) reported a family in which 9 of the 10 males in 2 generations (*i.e.*, 5 brothers and 4 of their nephews) manifested the clinical picture of seminiferous tubule dysgenesis; in 3 of these, testicular biopsy findings were consistent with the diagnosis, and also revealed a male chromatin pattern (2). Nelson and Heller (22) refer to 3 pairs of brothers in their group of patients with seminiferous tubule dysgenesis. Klotz and Sors (23) reported the syndrome as it occurred in first cousins, only 1 of whom is described in detail.

The present 2 patients as well as the other familial cases of seminiferous tubule dysgenesis can be invoked as indirect evidence in support of a genetic influence upon the development of this condition. Additional support for a genetic factor has been obtained by Stewart *et al.* (24) from their

FIG. 4. Testicular tissue, Case 2, showing sclerotic and hyalinized dysgenetic tubules. (Masson's trichrome ×223.)

analysis of childlessness in relatives of affected individuals. A familial incidence which appears to be more than that expected by chance alone is, therefore, the most convincing argument for the existence of a genetic mechanism in seminiferous tubule dysgenesis, although agreement is lacking as to the nature of the postulated defect (1, 2, 7, 8, 24, 25). It must be admitted that the present cases, in patients deriving from a single ovum, do not constitute a *"familial incidence" of seminiferous* tubule dysgenesis in the usual meaning of the term. However, the concordance of the twins with respect to this syndrome is in itself suggestive evidence for a genetic factor, as has been demonstrated for mongolism (26).

An additional, if less substantial, argument in favor of genetic determination depends on the occasional coexistence with seminiferous tubule dysgenesis of heritable disorders such as dystrophia myotonica (1, 20, 27). With the exception of an aunt with deaf-mutism, however, neither the twins nor their relatives bore any evidence of such defects.

An alternative theory to that of genetic determination of this syndrome has been proposed by Witschi, Nelson and Segal (9). They postulate that seminiferous tubule dysgenesis may be the result of defects in the unfertilized ovum (such as "overripeness") or perhaps of other deleterious influences on the developing embryo early in fetal life, any of which can produce (at least in amphibian analogues) failure of the germ cells during embryogenesis and resultant development of sterile testes in genetic females. Moreover, "overripeness" of the amphibian ovum has been shown by Witschi (28) to result in an increased incidence of twinning, in addition to sex reversal. On the other hand, a specific deleterious intra-uterine factor has not yet been implicated in the etiology of seminiferous tubule dysgenesis.

The available evidence would seem to lend support to the concept that a genetic factor is involved in the pathogenesis of seminiferous tubule dysgenesis. Although the conclusion is not warranted that this disorder is *genetically determined*, the evidence at the present time does lend weight to the hypothesis of *genetic predisposition* as an important factor in the etiology of this syndrome.

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REFERENCES

1. Grumbach, M. M.; Blanc, W. A., and Engle, E. T.: Sex chromatin pattern in seminiferous tubule dysgenesis and other testicular disorders: relationship to true

hermaphrodism and to Klinefelter's syndrome, with a review of gonadal ontogenesis, *J. Clin. Endocrinol. & Metab.* **17**: 703, 1957.

2. Grumbach, M. M., and Barr, M. L.: Cytologic tests of chromosomal sex in relation to sexual anomalies in man, *in* Recent Progress in Hormone Research, ed. by G. Pincus. New York, Academic Press Inc., 1958, vol. 14, pp. 255–334.
3. Klinefelter, H. F., Jr.; Reifenstein, E. C., Jr., and Albright, F.: Syndrome characterized by gynecomastia, aspermatogenesis without a-leydigism, and increased excretion of follicle-stimulating hormone, *J. Clin. Endocrinol.* **2**: 615, 1942.
4. Heller, C. G., and Nelson, W. O.: Hyalinization of the seminiferous tubules associated with normal or failing Leydig-cell function. Discussion of relationship to eunuchoidism, gynecomastia, elevated gonadotrophins, depressed 17-ketosteroids and estrogens, *J. Clin. Endocrinol.* **5**: 1, 1945.
5. Ferguson-Smith, M. A.; Lennox, B.; Mack, W. S., and Stewart, J. S. S.: Klinefelter's syndrome: frequency and testicular morphology in relation to nuclear sex, *Lancet* **2**: 167, 1957.
6. Bunge, R. G., and Bradbury, J. T.: Newer concepts of the Klinefelter syndrome, *J. Urol.* **76**: 758, 1956.
7. Danon, M., and Sachs, L.: Sex chromosomes and human sexual development, *Lancet* **2**: 20, 1957.
8. Hoffenberg, R., and Jackson, W. P. U.: Sex chromatin and intersex, *J. Clin. Endocrinol. & Metab.* **17**: 454, 1957.
9. Witschi, E.; Nelson, W. O., and Segal, S. J.: Genetic, developmental and hormonal aspects of gonadal dysgenesis and sex inversion in man, *J. Clin. Endocrinol. & Metab.* **17**: 737, 1957.
10. Plunkett, E. R., and Barr, M. L.: Testicular dysgenesis affecting the seminiferous tubules principally, with chromatin-positive nuclei, *Lancet* **2**: 853, 1956.
11. Bradbury, J. T.; Bunge, R. G., and Boccabella, R. A.: Chromatin test in Klinefelter's syndrome, *J. Clin. Endocrinol. & Metab.* **16**: 689, 1956.
12. Witschi, E.; Nelson, W. O., and Segal, S. J.: Genetic, developmental and hormonal aspects of gonadal dysgenesis and pseudohermaphrodism, (abstract) *J. Clin. Endocrinol. & Metab.* **16**: 922, 1956.
13. Grumbach, M. M.; Engle, E. T.; Blanc, W. A., and Barr, M. L.: The sex chromatin pattern in testicular disorders: relationship to pathogenesis and to true hermaphrodism, (abstract) *J. Clin. Endocrinol. & Metab.* **16**: 923, 1956.
14. Polani, P. E.; Hunter, W. F., and Lennox, B.: Chromosomal sex in Turner's syndrome with coarctation of the aorta, *Lancet* **2**: 120, 1954.
15. Grumbach, M. M.; Van Wyk, J. J., and Wilkins, L.: Chromosomal sex in gonadal dysgenesis (ovarian agenesis): relationship to male pseudohermaphrodism and theories of human sex differentatiation, *J. Clin. Endocrinol. & Metab.* **15**: 1161, 1955.
16. Barr, M. L.: The sex chromatin and its application to errors in sex development, *in* Modern Trends in Obstetrics and Gynaecology, ed. by Bowes, K. London, Butterworth & Co. Ltd., 1955, Series 2, pp. 117–128.
17. Nelson, W. O.: Sex differences in human nuclei with particular reference to the "Klinefelter syndrome," gonadal agenesis and other types of hermaphroditism, *Acta endocrinol.* **23**: 227, 1956.
18. Sohval, A. R., and Soffer, L. J.: Congenital familial testicular deficiency, *Am. J. Med.* **14**: 328, 1953.
19. Sohval, A. R.; Gabrilove, J. L.; Gaines, J. A., and Soffer, L. J.: Observations on the sex chromatin in testicular disorders, *J. Mt. Sinai Hosp.* **23**: 647, 1956.

20. NADLER, C. S.; STEIGER, W. A.; TRONCELLETI, M., and DURANT, T. M.: Dystrophia myotonica, with special reference to endocrine function (Klinefelter's syndrome), *J. Clin. Endocrinol.* **10**: 630, 1950.
21. REIFENSTEIN, E. C., JR.: Hereditary familial hypogonadism, *Proc. Am. Fed. Clin. Res.* **3**: 86, 1947.
22. NELSON, W. O., and HELLER, C. G.: Diseases of the reproductive system, *Annual Rev. Med.* **2**: 179, 1951.
23. KLOTZ, H. P., and SORS, C.: Remarques cliniques, histologiques et pathogéniques sur certaines atrophies testiculaires idiopathiques (type Klinefelter), *Semaine hôp. Paris* **30**: 4431, 1954.
24. STEWART, J. S. S.; FERGUSON-SMITH, M. A.; LENNOX, B., and MACK, W. S.: Klinefelter's syndrome: genetic studies, *Lancet* **2**: 117, 1958.
25. REIFENSTEIN, E. C., JR.: Quoted in reference 1, p. 725.
26. ALLEN, G., and BAROFF, G. S.: Mongoloid twins and their siblings, *Acta genet.* **5**: 294, 1955.
27. SIEBENMANN, R. E.: Symposia on nuclear sex, *Lancet* **2**: 637, 1957.
28. WITSCHI, E.: Overripeness of the egg as a cause of twinning and teratogenesis: a review, *Cancer Res.* **12**: 763, 1952.

CYTOLOGY OF HUMAN URINARY SEDIMENT: DIAGNOSTIC VALUE OF THE NON-NUCLEATED CELL*

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ABSTRACT

Epithelial cells of the distal urethra (studied in voided specimens of urine) respond in the same way to ovarian steroids as do the cells of the vaginal mucosa, throughout a normal menstrual cycle. Among these urinary cells, a non-nucleated, acidophilic amorphous structure termed the "non-nucleated cell" is present normally in the proportion of 1 to 20 per cent. When these non-nucleated structures are included in a count of 100 cells, striking variations are consistently observed in several pathologic conditions. We have confirmed the original observations of Galli Mainini that these non-nucleated elements are found in greatest abundance in urinary smears after castration. They are therefore an index of ovarian deficiency rather than of ovarian estrogenic stimulation. They are increased in ovarian dysgenesis, but reduced by estrogen therapy. Similarly, they are at a high level in cases of polycystic ovaries, but drop precipitately after wedge resection. The high level seen after oophorectomy in patients with breast cancer falls abruptly after hypophysectomy. In cases of anovulatory menstrual cycles, secondary amenorrhea or early pregnancy, the proportion of non-nucleated cells is low. It is postulated that, in the absence of functioning ovaries, the adrenals produce an estrogenic substance capable of supporting partial development of the urethral epithelium. With this support, the urethral cells are shed in a semi-degenerate state, without nuclei, but they do not revert to the wholly atrophic picture seen when the adrenal is also inactivated. It is felt that the changes in the level of the non-nucleated cells represent a significant compensatory mechanism of the adrenal in ovarian-deficient states. The percentile counts in 60 patients with various conditions are presented in support of these views.

CYTOLOGIC studies of human urine have recently been focused chiefly on the important finding that carcinoma sarising in the urologic tract may often be detected by their contribution of exfoliated cells to the urinary sediment (1, 2). Little attention has been paid to variations in the

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normal cell population washed downward in a voided specimen in the female. Since the squamous epithelium of the lower urethra develops embryologically as an upgrowth from the urogenital sinus in common with the vaginal mucosa, it is not surprising to find that changes in epithelial elements in the urine reflect ovarian activity in a way comparable to that seen in the vaginal smear (3). The same cell types are found in the stratified urethral epithelium and, with similar staining techniques, they can be recognized in the urine as reflecting not only states of ovarian deficiency but also hormonal changes during the normal menstrual cycle.

In 1956 Galli Mainini and associates called attention to a cellular element which he termed the "anucleated cell," found as a normal constituent in smears of urinary sediment (4). This non-nucleated structure stains deeply yellow to dark orange with heavy granular conglomerates. It is irregular in outline and varies greatly in contour, being smaller than a precornified or cornified cell and often having broken or wrinkled edges (Fig. 1). Its appearance suggests that of a poorly preserved, keratinized fragment that might be degenerated residual tissue from the fully cornified cell associated with maximum estrogenic stimulation. Because it looks like a fragmented particle, it has generally been disregarded as a bit of nonspecific debris by many cytologists when recording percentage counts of cell types in an evaluation of ovarian hormonal stimulation. When these cells are included in such a count, however, Galli Mainini has demonstrated that they are in greatest abundance, not in association with estrogen production but in association with states of ovarian deficiency, as postmenopausally and after x-ray castration. These findings raise the problem of their origin and significance. In the present investigation we have confirmed and extended the observations of Galli Mainini and co-workers. We suggest the use of the term "non-nucleated" to differentiate this cell from the large, yellow, cornified cell that has already lost its nucleus and that represents a high degree of estrogen production. The studies reported here indicate that incorporation of the non-nucleated cell count into a cytologic appraisal of urinary sediment lends further significance to this convenient diagnostic tool.

METHODS

Collection

The first morning specimen of urine is preferred, since it is unusually rich in cells; however, a specimen voided later in the day can be used. If it is necessary to keep the urine sample for longer than twelve hours before preparing the slides, $\frac{1}{3}$ volume of 95 per cent alcohol should be added, to prevent cell degeneration.

Technique of smear

One of 2 methods can be used to prepare the specimen for cytologic analysis.

1) The simpler procedure is that described by Galli Mainini *et al.* (5). With this

method, the urine is drawn by means of water suction through a rubber tube in which a small piece of cotton has been inserted. The cotton acts as a filter to entrap the cells. The cotton plug is removed after suctioning and pressed many times over a glass slide. The slide should previously have been prepared with a thin layer of egg albumin. After smearing it is immediately fixed in a solution containing equal parts of ether and 95 per cent alcohol. Two minutes is enough for fixation but the slide may be kept in the solution for many days without damage to the cells.

2) A standard amount of urine should be centrifuged from all specimens. The richness or paucity of cells may have diagnostic significance. A 50-cc. aliquot of urine is spun for ten minutes at 2,000 rpm. The sediment is aspirated and a drop or two spread evenly over a slide, which is then fixed as described for the first method.

Staining

All our preparations were stained for ninety seconds by the Shorr technique (6).

Reading

The number of cells, the general pattern, and the clumping and wrinkling may furnish important information regarding certain clinical conditions such as pregnancy, the phase of the normal menstrual cycle, and so on. It is therefore worth while to scan the slide first under low power in order to obtain a general impression of the field. Then, under high power, 100 cells are counted in at least 2 fields (or more), noting the percentages of the 5 varieties.

Classification of cells

In the sediment of normal urine there are 5 kinds of cells. Four of them can be named in the order in which they develop from the basal to the superficial layer, *i.e.*, basal, intermediate, precornified and cornified; the fifth is the "non-nucleated" cell. The first four groups are identical with those seen in the normal vaginal smear. Their number and distribution vary according to the phase of the menstrual cycle in normal females. Thus, in the urinary smear, the ratio of basal cells to precornified or cornified cells and the effect of progesterone are diagnostically as valid as in the vaginal smear. The "non-nucleated cell" is normally found in urinary sediment and has particular significance in certain pathologic endocrine states.

MATERIAL

Cytologic counts, including those of the non-nucleated cell, were made on smears of urine from subjects of both sexes from infancy to old age in both normal and pathologic states. We have limited this report, however, to the findings in 8 groups of women as follows:

1. Five normal women who had regular menstrual cycles.
2. Eight women surgically castrated for carcinoma of the breast and 5 women who had undergone a spontaneous menopause.
3. Four cases of ovarian dysgenesis.
4. Ten women who had been subjected to hypophysectomy after surgical castration for carcinoma of the breast.
5. Eleven cases diagnosed clinically as Stein-Leventhal syndrome.
6. Eight women with anovulatory cycles.
7. Twelve cases diagnosed clinically as secondary amenorrhea (variety of causes).
8. Ten women in the first trimester of pregnancy.

TABLE 1. NON-NUCLEATED CELLS IN THE URINE OF CASTRATED AND POSTMENOPAUSAL WOMEN

Patient No.	Age (yrs.)	Cells* of urinary smear (%)					Time after operation
		Ba.	Int.	Pco.	Co.	N. Nucl.	
Surgical Castrates (Ca. of breast)							
1	53	2	2	42	8	45	17 mos.
2†	38	3	26	20	0	51	20 mos.
3	36	3	20	20	10	47	56 mos.
4	40	2	30	21	3	44	44 mos.
5	47	5	40	42	0	13‡	22 days
6	44	2	20	35	0	43	9 mos.
7	40	29	11	4	8	48	24 mos.
8	53	6	11	10	8	65	9 mos.
9	45	2	38	15	2	43	10 mos.
Postmenopausal Women							
10	58	3	20	20	5	52	
11	64	5	30	20	5	40	
12	66	0	33	30	2	35	
13	65	2	40	20	3	35	
14	60	5	20	45	15	35	

* In this and subsequent tables:
Ba. = basal.
Int. = intermediate.
Pco. = precornified.
Co. = cornified.
N.Nucl. = non-nucleated.
† X-ray castration.
‡ Non-nucleated cells were low because the time between castration and the smear was short (22 days).

RESULTS

Day-by-day variations in the types of cells found in a voided urine specimen during a normal menstrual cycle have previously been adequately described by others (7–9). Our observations during 5 five cycles in normal young women confirm these findings. A typical example is shown in Figure 2. The peak of cornification is clearly seen to coincide with the time of ovulation, as indicated by a change from negative to positive pregnanediol excretion between days 14 and 16. The proportion of non-nucleated cells in the urinary smear during this cycle ranged from 2 to 10 per cent. In other normal cycles, the highest proportion noted for these cells was 20 per cent.

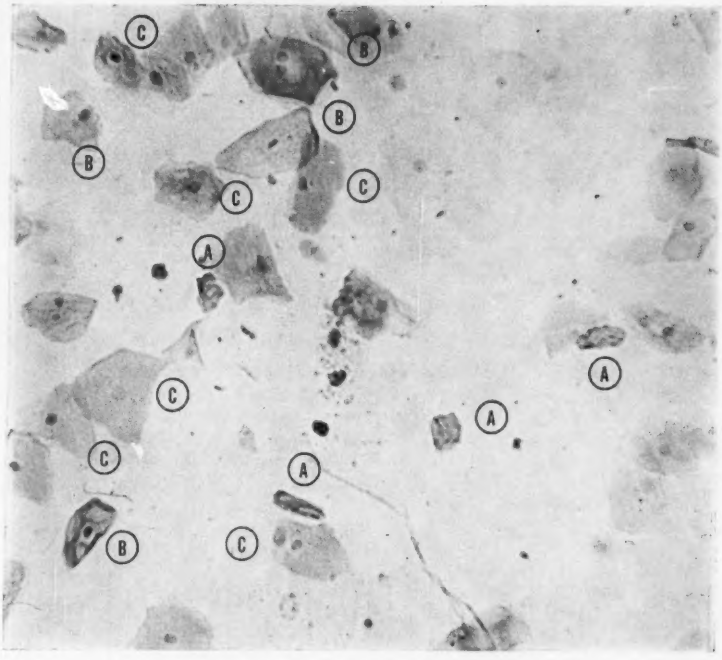

FIG. 1. Smear from a centrifuged voided specimen of urine. **A** = non-nucleated cells; **B** = cornified cells; **C** = precornified cells.

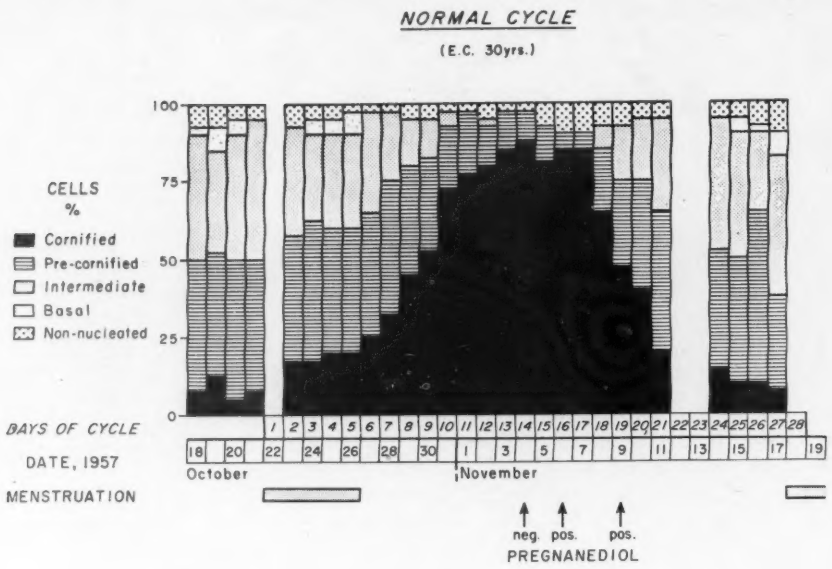

FIG. 2. Daily variations in cell types in a voided urine specimen throughout a normal menstrual cycle.

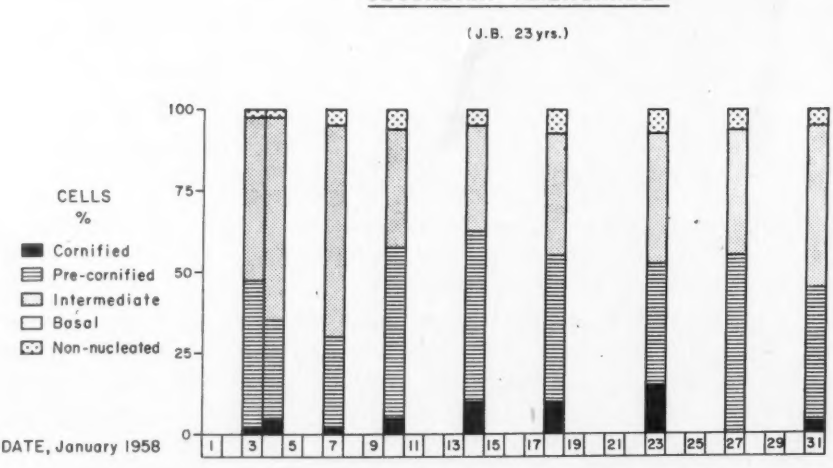

FIG. 3. Cell types in a voided urine specimen in a case of secondary amenorrhea treated by psychotherapy. The cornified cell count was never more than 15 per cent in this month—a percentage similar to that found at the lowest level of estrogen production in a normal cycle (Fig. 2); yet, in the presence of intact ovaries, the non-nucleated cell count was low.

The percentage of different cells found in smears of the urine from 8 castrated women and 5 postmenopausal women are shown in Table 1. All but 1 showed a high percentage of non-nucleated cells—far above the range found during the normal menstrual cycle. The exception was a patient who was examined only three weeks after castration. It is probable that in this case too short a time had elapsed for the non-nucleated cells to make their appearance.

In Table 2 are listed the percentages of cell types in 4 cases of ovarian dysgenesis. In all 4, the initial smears showed a percentage of non-nucleated cells that was higher than normal. When estrogen therapy was given in the last 2 cases, this percentage fell abruptly.

Table 3 shows the percentage of non-nucleated cells in the urine of 9 patients who underwent hypophysectomy for metastatic carcinoma of the breast. The postcastration prehypophysectomy smears in 8 of these are also listed in Table 1, but are repeated here to emphasize the contrast.

TABLE 2. NON-NUCLEATED CELLS IN THE URINE OF PATIENTS WITH OVARIAN AGENESIS

Patient No.	Age (yrs.)	Cells of urinary smear (%)										Duration of estrogen therapy (days)
		Without therapy					During estrogen therapy					
		Ba.	Int.	Pco.	Co.	N.Nucl.	Ba.	Int.	Pco.	Co.	N.Nucl.	
15	14	0	2	10	4	84						
		7	15	7	0	71						
		5	7	20	6	62						
		9	8	4	0	79						
		11	17	10	0	62						
16	15	5	3	53	8	31						
		14	22	27	6	31						
		4	6	15	9	66						
		14	10	32	6	38						
		5	5	23	1	66						
17	14	5	5	18	2	70	0	0	5	81	14	9
							0	3	15	82	0	10
							0	0	36	62	2	11
							0	1	5	90	4	12
							0	4	50	41	5	13
							0	5	27	61	7	14
							0	0	6	94	0	16
18	15	0	0	5	12	83	9	8	2	56	25	1
							0	4	6	72	18	2
							0	1	13	60	26	3

TABLE 3. NON-NUCLEATED CELLS IN THE URINE OF OOPHORECTOMIZED WOMEN BEFORE AND AFTER HYPOPHYSECTOMY

Patient No.	Cells of urinary smear (%)										No. of days after operation
	Before hypophysectomy					After hypophysectomy					
	Ba.	Int.	Pco.	Co.	N. Nucl.	Ba.	Int.	Pco.	Co.	N. Nucl.	
2	3	26	20	0	51	30	30	15	21	4	16
3	3	20	20	10	47	19	40	20	18	3	12
4	2	30	21	3	44	14	56	21	5	4	350
7	29	11	4	8	48	40	25	28	5	2	15
5	5	40	42	0	13*	24	50	26	0	0	14
6	2	20	35	0	43	3	30	38	20	9	6
9	2	38	15	2	43	6	43	30	16	5	15
8	6	11	10	8	65	18	34	26	20	2	16
19	2	15	13	3	67	10	14	70	5	1	10

* Period between oophorectomy and hypophysectomy was short (22 days).

After hypophysectomy in 8 patients, the previously elevated percentages fell abruptly to low levels—in 1 case as early as six days following ablation of the pituitary.

Eleven cases clinically diagnosed as Stein-Leventhal syndrome were studied (Table 4). In 6 of the 11, the diagnosis was subsequently confirmed at the time of ovarian wedge resection. In all instances, the percentage of non-nucleated cells in the urinary smear before operation was higher than the top level of the normal range in the controls, and after operation the high percentage of non-nucleated cells was sharply reduced. In some cases, this reduction occurred within a surprisingly short interval of only three or four days.

In 8 patients under study in the Clinic, the diagnosis of anovulatory cycles was based on the lack of pregnanediol in the urine throughout prolonged intervals between menstrual flows. In all but 2 cases the proportion of non-nucleated cells in the urinary smear was below 20 per cent (Table 5); and in these 2, higher counts were not consistently found.

Twelve women were under long-term endocrine study because of secondary amenorrhea. Several of them were undergoing psychiatric investigation. No organic disease had been demonstrated by a thorough endocrine work-up, including roentgenograms of the pituitary and determinations of urinary steroids. In only 4 was the proportion of non-nucleated cells in the urinary sediment occasionally as high as 20–30 per cent (Table 6).

In Table 7 is shown the incidence of the non-nucleated cell in smears of

TABLE 4. NON-NUCLEATED CELLS IN THE URINE OF WOMEN WITH THE STEIN-LEVENTHAL SYNDROME

Patient No.	Age (yrs.)	Cells of urinary smear (%)										No. of days after operation
		Before operation					After operation					
		Ba.	Int.	Pco.	Co.	N.Nucl.	Ba.	Int.	Pco.	Co.	N.Nucl.	
20	25	0	6	20	2	72	0	2	50	47	1	28
21	45	0	30	29	5	36	1	40	41	8	10	16
22	33	0	25	25	10	40	0	10	30	55	5	150
23	18	0	0	15	35	50	0	5	26	40	29	3
							0	16	28	42	14	4
							0	7	31	61	1	7
							0	8	52	40	0	8
24	29	0	1	49	16	34	0	6	58	19	17	4
							0	0	66	25	9	8
25	19	0	0	20	2	78						
26	23	0	6	40	10	44	3	7	55	28	7	3
							0	5	61	32	2	4
27	32	4	20	28	4	44						
28	35	3	30	25	10	32						
29	27	0	40	42	5	33						
30	20	2	25	20	3	50						

the urine from 10 normal women studied during the first trimester of pregnancy. The percentage of non-nucleated cells was invariably low.

DISCUSSION

Certain points of interest need emphasis in discussing variations in urinary cytology throughout a normal menstrual cycle, as seen in Figure 2. A few basal cells may be found at the time of the menses and for the first few days thereafter at the low point of estrogen production. Cornification increases relatively rapidly beginning on day 9 and maintains a high level from that time until, under the influence of progesterone, the percentage drops again on day 16. The luteal phase is characterized by a grouping of

TABLE 5. NON-NUCLEATED CELLS IN THE URINE OF WOMEN WITH ANOVULATORY MENSTRUAL CYCLES

Patient No.	Age (yrs.)	Date of urinary smear	Cells of urinary smear (%)				
			Ba.	Int.	Pco.	Co.	N.Nucl.
31	31	Mar. 6, '56	4	20	40	21	15
32	35	Apr. 18, '57	0	16	58	21	5
33	15	Apr. 20, '57	20	40	30	1	9
		Apr. 27, '57	12	11	54	7	16
		May 4, '57	0	28	21	35	16
		May 21, '57	3	8	67	15	7
34	17	May 24, '57	2	0	82	16	0
		May 27, '57	0	0	70	4	26
		June 7, '57	0	9	59	4	28
		June 14, '57	0	16	77	1	6
35	15	Sept. 6, '57	0	1	71	16	12
		Sept. 15, '57	1	1	25	56	17
36	41	Oct. 28, '57	0	0	72	26	2
		Nov. 5, '57	1	1	70	24	4
		Nov. 6, '57	1	9	41	46	3
		Nov. 8, '57	1	38	31	17	13
37	15	Mar. 7, '58	1	10	76	12	1
38	31	Nov. 14, '57	0	0	29	6	65
		Nov. 20, '57	1	13	64	8	14
		Nov. 27, '57	6	8	67	6	13
		Dec. 3, '57	0	22	50	10	18
		Dec. 10, '57	0	3	66	9	21
		Dec. 17, '57	0	25	35	15	25
		Dec. 23, '57	0	1	36	47	16
		Dec. 30, '57	0	2	18	77	3
		Jan. 10, '58	0	7	6	84	3

cells in sheets with some wrinkling of the cytoplasmic borders, and by the appearance of a modified intermediate cell (called the navicular cell because of its shape) which is typically present in early pregnancy. In the normal menstrual cycles we have studied, the proportion of non-nucleated cells in the urinary smear varies from 0 to 20 per cent—never higher.

Although a considerable variation in distribution of cell types will be found between subjects, each individual tends to show a characteristic pattern. Once this pattern has been established, then the typical changes

TABLE 6. NON-NUCLEATED CELLS IN URINE OF WOMEN WITH SECONDARY AMENORRHEA

Patient No.	Age (yrs.)	Date of urinary smear	Cells of urinary smear (%)				
			Ba.	Int.	Pco.	Co.	N.Nucl.
39	23	Dec. 9, '56	0	30	36	18	16
		Dec. 24, '56	0	37	50	9	4
		Dec. 26, '57	0	21	60	13	6
		Dec. 31, '56	0	30	50	12	8
40	29	Dec. 7, '56	0	30	45	10	15
		Dec. 9, '56	0	45	45	7	3
		Jan. 3, '57	0	25	55	14	6
		Jan. 15, '57	0	35	53	10	2
41	32	Dec. 23, '56	0	60	20	16	4
		Jan. 6, '57	0	45	45	10	0
		Jan. 11, '57	0	36	50	7	7
		Jan. 21, '57	0	35	45	13	7
42	25	Feb. 14, '57	0	40	32	14	14
		Feb. 17, '57	0	30	43	15	12
43	36	Feb. 27, '57	0	40	47	9	4
		Mar. 6, '57	0	50	40	3	7
44	28	Dec. 6, '57	0	50	43	4	3
		Jan. 30, '57	0	36	50	9	5
		Feb. 15, '57	0	40	47	8	5
		Feb. 17, '57	0	20	45	13	22
45	48	Apr. 26, '57	0	38	20	15	27
46	20	Mar. 25, '58	0	33	60	6	1
47	32	May 18, '57	0	30	67	3	0
		May 26, '57	0	2	75	17	6
		July 25, '57	1	6	67	26	0
48	34	May 31, '57	0	0	82	5	13
		June 1, '57	0	0	84	3	13
		June 2, '57	0	0	78	12	10
		June 3, '57	0	1	86	2	11
49	18	Oct. 28, '57	0	22	47	8	23
50	22	Jan. 18, '57	2	28	40	10	20
		Jan. 26, '57	13	17	59	1	10

caused by progesterone may be used with some accuracy (as in the vaginal smear) for the dating of ovulation. The urinary smear has the obvious added advantage, particularly in children and adolescents, that it can be obtained without vaginal manipulation and consequent hazard of undesirable emotional trauma.

Galli Mainini and associates (4) reported an increase in non-nucleated cells following x-ray castration and postmenopausally. Our observations on 8 women surgically castrated and on 5 postmenopausal women aged 60 or more confirmed these findings (Table 1), and point to estrogen deprivation due to ablation or elimination of previously functioning ovaries as the

TABLE 7. NON-NUCLEATED CELLS IN THE URINE IN CASES OF NORMAL PREGNANCY

Patient No.	Cells of urinary smear (%)					Weeks of pregnancy
	Ba.	Int.	Pco.	Co.	N.Nucl.	
51	0	22	61	8	9	6
52	0	40	40	11	9	6
53	0	50	49	0	1	6
54	0	30	59	5	6	8
55	0	40	53	5	2	8
56	0	40	57	3	0	6
57	7	13	76	3	1	7
58	5	3	89	3	0	8
59	0	31	67	1	1	7
60	0	50	42	7	1	7

chief etiologic factor in the increased percentage of non-nucleated cells in the urine. In these cases the pituitary responds with increased production of gonadotropin. High titers of gonadotropin are also found in cases of ovarian dysgenesis. In these patients there is no sudden withdrawal of estrogen, since they have never possessed any functioning ovarian tissue. The finding of an abnormally high percentage of non-nucleated cells (see Table 2) which drops precipitately (along with a fall in FSH excretion) during adequate estrogen therapy might suggest an association with excess pituitary gonadotropins. This concept is strengthened by our observations on castrated women who underwent hypophysectomy. The non-nucleated cells almost disappeared from the urine, whereas the basal and intermediate cells were present in abundance (Table 3). Excessive pituitary gonadotropins cannot be directly implicated, however. In all urine specimens from normal male subjects and from some of the prepuberal girls thus far

studied, we noted a high percentage of non-nuclear cells although the excretion of pituitary gonadotropins was at normal or subnormal levels.

From these considerations we have concluded that it is neither estrogen deprivation nor a direct effect of excessive gonadotropins that leads to increased numbers of this peculiar cell in the urine. Rather, it appears possible that in the absence of ovarian estrogen, some other endocrine gland provides sufficient stimulation to prevent complete atrophy (as after hypophysectomy) and to develop the squamous urethral epithelium to a point where cells are cast off in the stage of "non-nucleated" semi-degeneration.[1] Earlier investigators (4, 10) postulated that the adrenal might exert such an estrogen-like effect. For this reason, we studied some cases of Stein-Leventhal syndrome, which often presents clinical features suggesting adrenal hyperactivity. Indeed, the hirsutism, obesity and amenorrhea in these patients may simulate closely the same set of symptoms in the adrenogenital syndrome. In all the cases of Stein-Leventhal syndrome there was an increase in non-nucleated cells and a low degree of cornification (Table 4). It is of particular interest that the operation of wedge resection in 6 of these cases was followed almost immediately by a pronounced reduction in the percentage of non-nucleated cells. The drop was from 44 to 2 per cent in one case (No. 26) within four days, and from 50 to 1 per cent in another case (No. 23) within seven days. Whether or not this operation may influence adrenal as well as ovarian function is a question that has yet to be solved. We have not had the opportunity to study well defined cases of primary adrenal hyperactivity. We suspect that if the ovary is physiologically intact, the influence of the adrenal in relation to the incidence of non-nuclear cells in the urine is not significant.

Since either irregular bleeding, oligomenorrhea or amenorrhea is characteristic of patients with Stein-Leventhal syndrome, it seemed worth while to check a group of patients in whom the diagnosis of anovulatory cycles had been established. In Table 5 it is seen that only rarely was the proportion of non-nucleated cells higher than the 20 per cent considered to be the top limit during normal menstrual cycles. In 1 case (No. 34) there was a variation from 0 to 28 per cent. It is possible that an occasional finding of higher-than-normal levels, particularly in young girls with some hirsutism and amenorrhea, may indicate the early development of a poly-

[1] Another case of particular interest is not included in these tables. The patient underwent hypophysectomy for metastatic carcinoma of the breast, during the fifth month of pregnancy. The ovaries were not removed. At time of delivery two months later, in the absence of the pituitary, a high level (85 per cent) of non-nucleated cells was found in the urinary smear, but after delivery the proportion dropped to 9 per cent. This case is to be reported in detail elsewhere (Jessiman, A., and Eglin, J.: Inactivation of antidiuretic activity of vasopressin during pregnancy, (*in press*)).

cystic condition of the ovaries. In such cases, the urinary smears should be followed with great care over the course of many months.

The group of patients with anovulatory bleeding (Table 5) were studied because they showed none of the clinical characteristics of the "Stein-Leventhal-adrenal" patients. Obviously, the irregular bleeding episodes documented the production of ovarian estrogens.

For comparison, we checked a group of 12 patients who had prolonged secondary amenorrhea from a variety of ill defined causes (Table 6). Complete endocrine studies had been made and no organic disease had been found. Several of these patients had been undergoing prolonged psychiatric therapy for what was believed to be a profound disturbance in the emotional sphere, leading to the amenorrhea. In none of these 12 cases was there an elevated percentage of non-nuclear cells in the urine. In Figure 3 it may be seen that over the course of a month there was very little day-to-day variation in the cytologic pattern in 1 of these patients. Even though there was a low degree of cornification and obviously low ovarian function, the estrogen produced by these ovaries apparently was sufficient to maintain an equilibrium with the pituitary and support a relatively normal cytologic picture without an abnormal proportion of non-nuclear cells.

Pregnant women in the first trimester (Table 7) were found not to have a high level of non-nucleated cells, although the test was made at a time when the production of chorionic gonadotropin was close to its peak. This contrasts with a previous finding (4) that administration of chorionic gonadotropin caused an increase in non-nucleated cells. We tested a few women in the last trimester of pregnancy, and did not find an increase in these cells.

In summary, the evidence from this study suggests that when the ovary is physiologically abnormal, non-functional or absent, an intact pituitary can stimulate the adrenal to produce an estrogen-like steroid which prevents complete atrophy of the urethral epithelium. The exfoliated cells in the urine are neither completely atrophic (basal) nor fully cornified, as the epithelium has developed only to an intermediate phase under this adrenal-induced "estrogen" stimulation. In this phase, the cells are washed off the epithelium when they have already lost their vitality and their nucleus. There is no doubt, however, that this represents a definite state of hormonal support, since after hypophysectomy when the adrenal is rendered completely inactive, these cells disappear from the urinary sediment.

REFERENCES

1. Staff, Vincent Memorial Laboratory: The Cytologic Diagnosis of Cancer. Philadelphia, W. B. Saunders Co., 1950, p. 147.
2. Papanicolaou, G. N.: Cytology of urine sediment in neoplasms of the urinary tract, *J. Urol.* **57**: 375, 1947.

3. SCHILLER, W.; Embryology and development of the female reproductive tract, *in* Progress in Gynecology, ed. by J. V. Meigs and S. H. Sturgis. New York, Grune & Stratton, 1950, vol. II, p. 1.
4. GALLI MAININI, C.; ABIZANO, C., and AMBRAD, N.: El extendido del sedimento de orina como medio dianostico, *Semana méd.* **108**: 351, 1956.
5. GALLI MAININI, C., and ABIZANO, C.: El extendido coriado del sedimendo de orina, *Semana méd.* **108**: 326, 1956.
6. SHORR, E. A.: A new technique for staining vaginal smears. III. A single differential stain, *Science* **94**: 545, 1941.
7. LENCIONI, L. J., and STAFFIERI, I. J.: Observations on urinary sediment cell counts in women, *Acta endocrinol.* **16**: 270, 1954.
8. LENCIONI, L. J.: Observaciones sobre el urocitograma, *Obst. y ginec. latino-Am.* **13**: 90, 1955.
9. ROFE, P.; The cells of normal human urine, *J. Clin. Path.* **8**: 25, 1955.
10. KLAUS, H.: Effects of hypophysectomy on FSH levels and vaginal cornification, *Surg. Forum* (Am. Coll. Surg.) **8**: 495, 1957.

VIRILIZING ADRENAL ADENOMA ASSOCIATED WITH INCREASED URINARY EXCRETION OF ESTROGENS AND 17-KETOSTEROIDS. ISOLATION OF STEROIDS FROM THE TUMOR

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ABSTRACT

A 34-year-old woman with an adrenocortical adenoma had hypertrichosis of the extremities, abdomen and face, clitoral hypertrophy, a small uterus and amenorrhea. There was no hypertonia. Roentgenologic examination (intravenous pyelogram) showed the left kidney to be lower than normal. A pneumoretroperitoneal roentgenogram showed a suprarenal tumor on the left side.

Steroid hormone metabolism was markedly disturbed. There was increased urinary excretion of 17-ketosteroids and of estrogens. The chromatographic differentiation and the absorption spectrum of the urinary 17-ketosteroids showed a predominant excretion of dehydroepiandrosterone. The ACTH-stimulation test revealed no rise of the excreted 17-ketosteroids, but an increase of the excreted 17-hydroxycorticoids and estrogens. The administration of 9α-fluorohydrocortisone caused only a slight depression of the 17-ketosteroids and estrogens. No sign of an estrogen effect was found in the vaginal smears.

After removal of the adrenocortical tumor, the urinary hormone levels immediately decreased to normal values. Facial hair diminished, and five weeks after operation spontaneous menstruation occurred. Since then the patient has menstruated regularly.

The tumor weighed 540 grams and was examined histologically and chemically. Small amounts of aldosterone, androsterone, Δ^4-androsten-3,17-dione, cortisone, dehydroepiandrosterone, estradiol, estrone and hydrocortisone were isolated from the tumor.

THIS report presents an interesting case of adrenal tumor accompanied by increased urinary excretion of estrogens and 17-ketosteroids. We had the opportunity to make thorough clinical and hormonal investigations, and to isolate various steroids from the tumor.

CASE REPORT

Anamnesis

The patient, a dressmaker aged 34, had menstruated regularly since the menarche at age 13. She had always been very healthy, had undergone no operations, and was

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* Director: Prof. Dr. Th. Koller.

† Director: Prof. Dr. H. Ludwig.

never pregnant. She was fully capable of work when entering the clinic in July 1957. Five years previously oligomenorrhea and hirsutism had been observed, and both had steadily increased. For two years she had had amenorrhea. Sexual libido was normal.

Clinical findings

Examination of the muscular patient revealed hypertrichosis of the extremities and abdomen and a marked growth of hair on the face (Fig. 1). The uterus was small, the vagina normal and the clitoris hypertrophied (Fig. 2). There was no hypertonia and no osteoporosis. The roentgenologic findings[1] (intravenous pyelogram) showed the right kidney to be in a normal position, whereas the left kidney was lower (Fig. 3). A pneumoretroperitoneal roentgenogram indicated a grapefruit-sized suprarenal tumor on the left side, which was not palpable even by examination under narcosis (Fig. 4).

General laboratory data

No abnormality was discovered by chemical and microscopic examination of the urine or by examination of the blood picture. The results of blood chemical analyses were normal, apart from a transient elevation of the serum bilirubin level (1.5 mg. per 100 ml.). The levels of blood sugar and of serum nonprotein nitrogen, urea, total protein, sodium, potassium, calcium, sodium chloride, cholesterol, phosphorus, phosphatase, protein-bound iodine, and iron were within the normal range, as were also the results of thymol-turbidity and gamma globulin-turbidity tests. Analysis by paper chromatography of the amino acids yielded normal values. A normal fall of eosinophils was observed after stimulation by ACTH.

CHEMICAL AND HISTOLOGIC FINDINGS

Hormone determinations

Table 1 summarizes the results of urinary hormone assays. The methods used for hormone analyses are described in the Appendix of this report.

The result of the chromatographic differentiation of the excreted neutral 17-ketosteroids is represented in Figure 5. The constituents of the different fractions were:

Fraction A (A_1+A_2):	artifacts and unknown 17-ketosteroids.
Fraction B:	dehydroepiandrosterone and epiandrosterone.
Fraction C:	androsterone, Δ^5-androstene-ol-(3α)-one-17, etiocholane-ol-(3α)-one-17, and Δ^9-etiocholene-ol-(3α)-one-17.
Fraction D:	11-hydroxyandrosterone and 11-hydroxyetiocholane-ol-(3α)-one-17.

The absorption spectrum of the reaction product of a urinary extract with Allen's reagent showed the typical maxima of dehydroepiandrosterone in comparison with a pure specimen of this compound (Fig. 6).

In order to investigate more clearly whether the adrenal abnormality

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[1] The roentgenologic examinations were carried out by Dr. K. Mainardus.

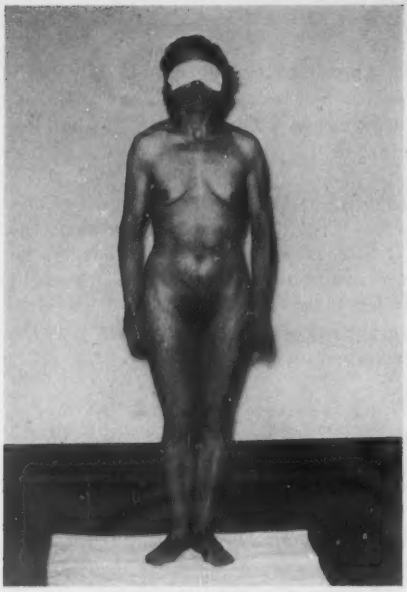

FIG. 1

FIG. 2

FIG. 3

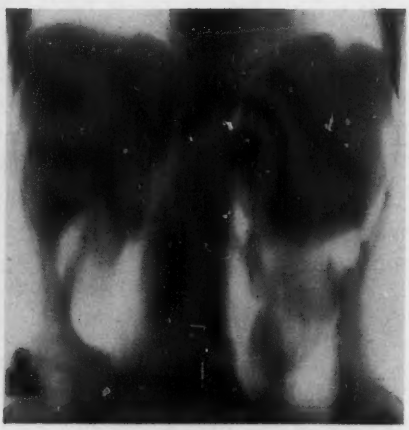

FIG. 4

FIG. 1. Front view of patient, showing hypertrichosis.

FIG. 2. Hypertrophied clitoris.

FIG. 3. Intravenous pyelogram, showing left kidney lower than right.

FIG. 4. Pneumoretroperitoneal roentgenogram, showing suprarenal tumor on the left side.

TABLE 1. URINARY HORMONE ASSAYS

	Urinary excretion per 24 hours	Normal range per 24 hours
17-Ketosteroids	200 mg. and 228 mg.	7 to 17.5 mg.
Estrogens (chemical method)		
estrone-estradiol	535 I.U.	50 to 450 I.U.
estriol	790 I.U.	50 to 500 I.U.
Estrogens (bioassay)	160 R.U.	25 to 125 R.U.
17-Hydroxycorticoids	17.0 mg.	4 to 10 mg.
Pregnanediol-pregnanetriol complex	0.6 mg.	2 to 13 mg.
Gonadotropin	less than 10 mouse uterus units	10 to 50 mouse uterus units

Estrone, estradiol, estriol and 2 unidentified phenolsteroids also were demonstrated by paper chromatographic analysis.

Papanicolaou test on vaginal smears: No estrogen effect; only basal cells.

was a tumor or a hyperplasia of the cortex, the effect of ACTH and of 9α-fluorohydrocortisone on steroid excretion was studied. The results are shown in Tables 2 and 3 and in Figure 7.

TABLE 2. ACTH-STIMULATION TEST

The patient was stimulated on each of two consecutive days with 25 units of ACTH in 1,000 ml. of a 5% glucose solution (intravenously) during eight hours.

	Urinary excretion per 24 hours			
	17-Ketosteroids (mg.)	17-Hydroxycorticoids (mg.)	Estrone + Estradiol (I.U.)	Estriol (I.U.)
Control day	188	17.0	370	670
First day of ACTH-stimulation	184	32.8	650	750
Second day of ACTH-stimulation	154	35.9	760	790
First day after ACTH	157	21.6	350	650
Second day after ACTH	147	4.1	590	510

TABLE 3. TEST WITH 9α-FLUOROHYDROCORTISONE (FLUORO-F)

The patient was given 15 mg. of fluoro-F daily by mouth (5 mg. every eight hours) on each of three consecutive days.

	Urinary excretion per 24 hours			
	17-Keto-steroids (mg.)	17-Hydroxy-corticoids (mg.)	Estrone +Estradiol (I.U.)	Estriol (I.U.)
Control day	147*	4.1*	590	510
First day of fluoro-F	92	9.8	440	350
Second day of fluoro-F	133	28.2	530	490
Third day of fluoro-F	125	25.5	610	500
Day after fluoro-F	153	9.5	590	340

* This test was made soon after the ACTH-stimulation test. The control day corresponds with the second day after ACTH (Table 2), which explains the low starting values for 17-ketosteroids and 17-hydroxycorticoids.

Diagnosis

A summary of the results of the hormone determinations indicated pronounced hyperfunction of the adrenal cortex, which was causing distinct changes in steroid metabolism. The unusually high production of androgen and estrogen had apparently suppressed secretion of gonadotropin by the pituitary gland, and therefore ovarian function had ceased. Chromatography and absorption spectra clearly showed that the compounds of fraction B (consisting mainly of the β-steroids, dehydroepiandrosterone and epiandrosterone) were, in particular, excreted in increased quantities. The source of these compounds is the adrenal cortex. Experience has proved that an absolute and relative increase of fraction B is mostly to be found in cases of adrenal tumor. The ACTH-stimulation test revealed no rise of the urinary 17-ketosteroids, but an increased excretion of 17-

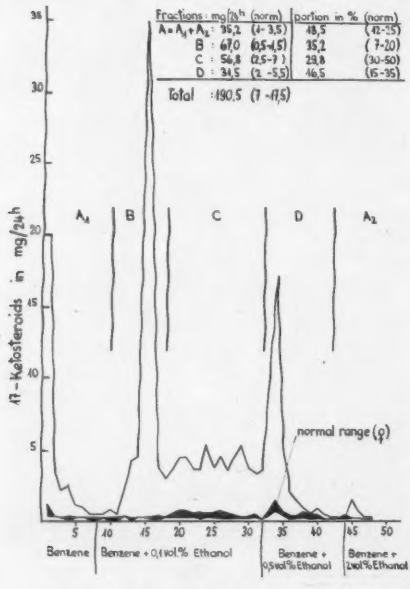

FIG. 5. Chromatographic differentiation of the urinary 17-ketosteroids before removal of adrenal tumor.

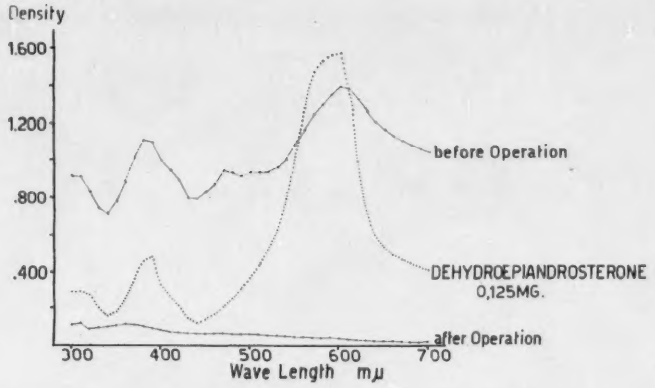

FIG. 6. Absorption spectra (before and after removal of adrenal tumor) of the reaction products of the urinary extracts with Allen's reagent, in comparison with pure dehydroepiandrosterone.

hydroxycorticoids and estrogens. The administration of 9α-fluorohydrocortisone caused only a slight depression of 17-ketosteroid and estrogen excretion. The results of the hormone determinations together with the anamnesis and the x-ray and clinical findings proved the presence of an adrenal tumor.

The operation was performed by Prof. H. Heusser (II Department of Surgery of the University of Basle). *A well defined tumor of the left adrenal was removed.*

Postoperative findings

There were no postoperative complications. Blood pressure and electrolyte concentration remained completely normal. The urinary hormone values immediately decreased to normal after removal of the tumor. Repetition of the chromatographic differentiation of the neutral 17-ketosteroids and of the absorption spectrum showed a marked decrease of the compounds of fraction B (dehydroepiandrosterone) (Figs. 6 and 8). Growth of hair on the extremities and facial hair diminished, and five weeks after operation menstruation spontaneously occurred. Since then the patient has menstruated regularly.

Histologic examination of the tumor

The tumor was 13 centimeters in diameter and weighed 540 grams. The histologic examination was carried out by Prof. S. Scheidegger in the Department of Pathological Anatomy of the University of Basle (Director: Prof. Dr. A. Werthemann). The report described the material taken from the cortical region as being brownish-yellow, strongly blood stained,

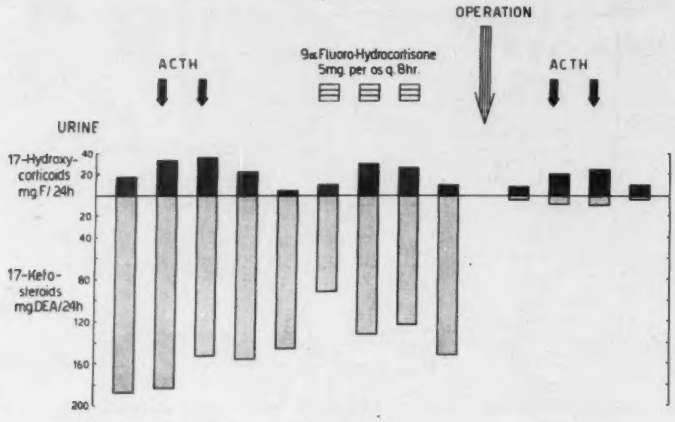

FIG. 7. The effect of ACTH and 9α-fluorohydrocortisone (fluoro-F) on the excretions of 17-hydroxycorticoids and 17-ketosteroids.

fibrous tissue with marked intercalations of adipose and connective tissue. There were small localized, glassy, reddish areas. Material taken from the medullary region was yellowish, marrowy soft, and rich in lipoid content. Figure 9 shows a histologic section from the cortical region.

Diagnosis: Differentiated, benign adenoma of the adrenal cortex.

Chemical examination of the tumor

The tumor was immediately extracted in our laboratories by a method proved good for adrenal cortex investigations. The extract was examined

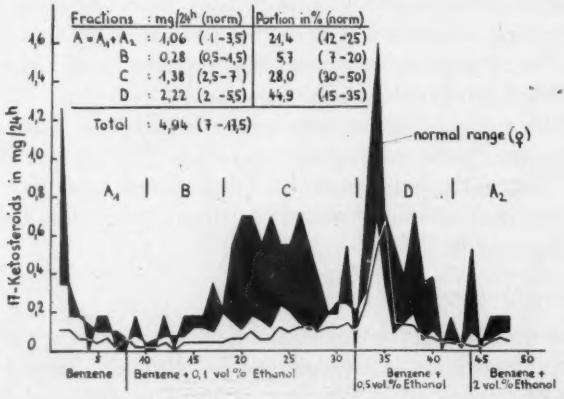
Fractions : mg/24h (norm) | Portion in % (norm)
A = A1 + A2 : 1,06 (1-3,5) | 21,4 (12-25)
B : 0,28 (0,5-1,5) | 5,7 (7-20)
C : 1,38 (2,5-7) | 28,0 (30-50)
D : 2,22 (2-5,5) | 44,9 (15-35)
Total : 4,94 (7-17,5)
normal range (♀)
17-Ketosteroids in mg/24h
A1
B
C
D
A2
Benzene
Benzene + 0,1 vol % Ethanol
Benzene + 0,5 vol % Ethanol
Benzene + 2 vol % Ethanol

FIG. 8. Chromatographic differentiation of the urinary 17-ketosteroids after removal of adrenal tumor.

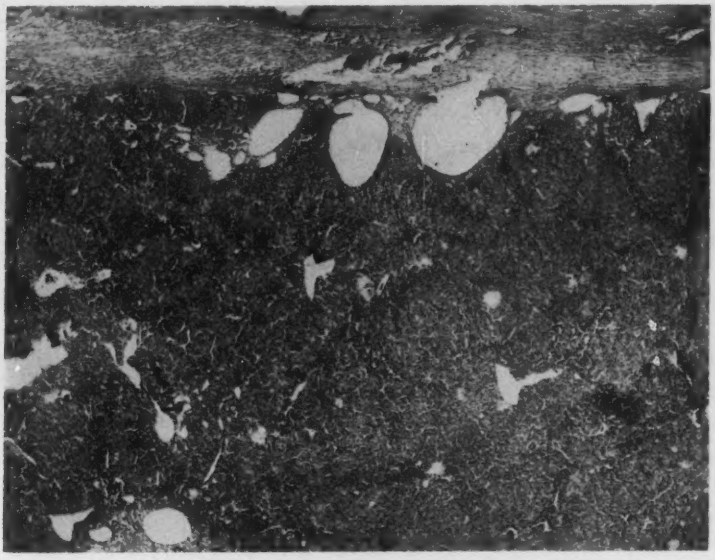

FIG. 9. Histologic section from the cortical region of the tumor (35×).

for steroids by paper chromatographic analysis. The experimental set-up for the chemical extraction of the tumor and the identification of the various steroids is described in the Appendix of this report. Only extremely small quantities of steroid compounds (calculated for the whole 540-gram tumor) were discovered. The various substances recovered were:

Compound	*Approx. amount (micrograms)*
Aldosterone	2
Androsterone	4
Δ^4-Androsten-3,17-dione	10
Cortisone	8
Dehydroepiandrosterone	8
Estradiol	5
Estrone	7
Hydrocortisone	5

DISCUSSION

Our investigations showed that the adrenocortical adenoma in this patient produced considerable amounts of chemically identifiable androgens and estrogens. The result of the chromatographic differentiation of the urinary 17-ketosteroids was very helpful in diagnosis. The observed excessive amounts of dehydroepiandrosterone (35.2 per cent of the total

17-ketosteroid excretion) indicated the presence of an adrenocortical tumor. In cases of hyperplasia of the cortex we have always found the percentile proportions of the excreted fraction groups (A, B, C and D) practically normal (1).

The relatively high urinary excretion of estrogens by this woman is noteworthy.

Recently West *et al.* (2) surveyed the literature reports concerned with adrenocortical hyperfunction. According to their survey, some patients with adrenocortical hyperfunction excrete large amounts of estrogens in the urine, whereas others excrete normal or decreased amounts. Frank (3) reported high levels of estrogenic material in the urine of patients with adrenocortical carcinoma, but low or normal levels in those with benign cortical hyperplasia or adenoma. He suggested that the level of urinary estrogens might be used to distinguish between malignant and benign cases. Other publications demonstrated that not all patients with adrenocortical carcinoma excrete excessive amounts of estrogens (4–10). Furthermore, patients with benign adrenocortical hyperplasia and adenoma may also excrete large quantities of estrogens (11–13). In nearly all of these investigations the urinary estrogens were determined by biologic methods and, with only a few exceptions, were not isolated and identified chemically. Mason and Kepler (14) were successful in isolating crystalline estrone from the urine of a woman with an adrenocortical carcinoma, but they failed to find any estradiol and estriol. West and his co-workers (2) isolated and identified relatively large amounts of estrone and estriol from the urine of a 58-year-old postmenopausal woman with metastatic adrenocortical carcinoma, by means of countercurrent distribution and paper chromatography. They observed in their case increased estrogen excretion after ACTH stimulation and suggested that these estrogens or their precursors were produced by the adrenocortical carcinoma and that this function of the tumor tissue was not autonomous.

In our case we also stimulated the patient with ACTH and observed an increase of estrogen and 17-hydroxycorticoid excretion (Table 2). The administration of 9α-fluorohydrocortisone caused a slight depression of the estrogen and 17-ketosteroid values (Table 3). Since our patient had intact ovaries and normal adrenocortical tissue as well as tumor tissue, it is impossible to be quite certain that the tumor was the only source of the urinary estrogens. The increase of urinary estrogens stimulated by the administration of ACTH showed that certainly adrenocortical tissue was involved. Since we know that normal adrenal glands secrete only small amounts of estrogens, we can conclude that the excessive excretion of estrogen could be accounted for mainly by the tumor tissue. The problem, whether or not the urinary estrogens were true secretory products of the

adrenal adenoma or metabolites of some other precursors, like androgens, remained. The isolation and paper chromatographic identification of small amounts of estrone and estradiol in the tumor material proved that estrogens were being secreted by the adenoma. Judging by our findings, the tumor apparently was not completely autonomous in its production of hormones. We were able to demonstrate that steroid excretion was altered by the administration of ACTH and 9α-fluorohydrocortisone.

The amounts of steroid compounds detected in the tumor material were extremely small. The isolation and identification of estrone and estradiol in the tumor extract is especially noteworthy.

Acknowledgments

The authors wish to acknowledge with gratitude the help and advice given by Prof. Dr. H. Heusser, Prof. Dr. Th. Koller, Prof. Dr. T. Reichstein, Prof. Dr. S. Scheidegger, Prof. Dr. R. Wenner and Dr. K. Meinardus. They also wish to express their appreciation to Mrs. R. Berde, Miss H. Hertig, Miss E. Huber, Miss L. Steigmeir and Mr. F. Waldmeier for their excellent technical assistance.

REFERENCES

1. Keller, M., and Hauser, A.: Der klinische Wert der chromatographischen Fraktionierung der neutralen 17-Ketosteroide in der Gynäkologie, *Arch. f. Gynäk.* **190**: 241, 1958.
2. West, C. D.; Damast, B., and Pearson, O. H.: Urinary estrogen excretion in Cushing's syndrome, *J. Clin. Endocrinol. & Metab.* **18**: 15, 1958.
3. Frank, R. T.: A suggested test for functional cortical adrenal tumor, *Proc. Soc. Biol. & Med.* **31**: 1204, 1933.
4. Cahill, G. F., and Robinson, J. N.: Androgenic tumors of the adrenal cortex in children: a report of four cases, *J. Urol.* **61**: 680, 1949.
5. Anderson, A. F.; Hain, A. M., and Patterson, J.: A case of adrenal carcinoma and its hormone diagnosis, *J. Path. & Bact.* **55**: 341, 1943.
6. Hain, A. M.: Adrenal tumors and pseudohermaphroditism: a hormone study of cases, *J. Path. & Bact.* **59**: 267, 1947.
7. Pratt, J. P., and Schaeffer, R. L.: Sex precocity, virilism, adrenal cortical tumor, *Am. J. Obst. & Gynec.* **49**: 623, 1945.
8. Walters, W., and Kepler, W. J.: Surgical lesions of the adrenal gland, *J.A.M.A.* **111**: 1061, 1938.
9. Kepler, E. J., and Keating, F. R., Jr.: Diseases of the adrenal glands. II. Tumors of the adrenal cortex, diseases of the adrenal medulla and allied disturbances, *Arch. Int. Med.* **68**: 1010, 1941.
10. Kenyon, A. T.; Gallagher, T. F.; Peterson, D. H.; Dorfman, R. I., and Koch, F. C.: The urinary excretion of androgenic and estrogenic substances in certain endocrine states. Studies in hypogonadism, gynecomastia, and virilism, *J. Clin. Invest.* **16**: 705, 1937.
11. Migeon, C. J., and Gardner, L. I.: Urinary estrogens (measured fluorometrically and biologically) in hyperadrenocorticism; influence of cortisone, compound F, compound B and ACTH, *J. Clin. Endocrinol. & Metab.* **12**: 1513, 1952.
12. Saphir, W., and Parker, M. L.: Adrenal virilism, *J.A.M.A.* **107**: 1286, 1936.

13. KELLER, M.: Increased urinary estrogen and 17-ketosteroid excretion associated with adrenal adenoma. Isolation of cholestane-dione-3,6 from the tumor, *J. Clin. Endocrinol. & Metab.* **16**: 1075, 1956.
14. MASON, H. L., and KEPLER, E. J.: Isolation of steroids from the urine of patients with adrenal cortical tumors and adrenal cortical hyperplasia, *J. Biol. Chem.* **161**: 235, 1945.
15. DREKTER, I. J.; HEISLER, A.; SCISM, G. R.; STERN, S.; PEARSON, S., and MCGAVACK, T. H.: The determination of urinary steroids. I. The preparation of pigment-free extracts and a simplified procedure for the estimation of total 17-ketosteroids, *J. Clin. Endocrinol. & Metab.* **12**: 55, 1952.
16. LORAINE, J. A., and BROWN, J. B.: Further observations on the estimation of urinary gonadotropins in non-pregnant human subjects, *J. Clin. Endocrinol. & Metab.* **16**: 1180, 1956.
17. WENNER, R.; KELLER, M., and HAUSER, G. A.: Sexualhormonausscheidung bei drohendem Partus immaturus und praematurus, *Gynaecologia* **143**: 159, 1957.
18. FORSHAM, P. H.; DI RAIMONDO, V.; ISLAND, D.; RINFRET, A. P., and ORR, R. H.: Dynamics of adrenal function in man, *in* The Human Adrenal Cortex, *Ciba Fndt'n. Colloq. Endocrinol.* **8**: 279, 1955.
19. DINGEMANSE, E.; HUIS IN'T VELD, L. G., and HARTOGH-KATZ, S. L.: Clinical method for the chromatographic-colorimetric determination of urinary 17-ketosteroids. II. Normal adults, *J. Clin. Endocrinol. & Metab.* **12**: 66, 1952.
20. ALLEN, W. M.; HAYWARD, S. J., and PINTO, A.: A color test for dehydroisoandrosterone and closely related steroids, of use in the diagnosis of adrenocortical tumors, *J. Clin. Endocrinol. & Metab.* **10**: 54, 1950.
21. BUSH, I. E.: Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissue, *Biochem. J.* **50**: 370, 1952.
22. ROHR, O.; HEUSSER, H.; ANLIKER, R., and RUZICKA, L.: II. Ueber die Bestimmung von Steroid-Hormonen in Tumor-Geweben. Dissertation (Rohr, O.), Eidgenössische Technische Hochschule in Zürich, Switzerland, 1956.
23. AXELROD, L. R.: The chromatographic fractionation and identification of compounds related to the estrogens, *Recent Progr. Hormone Res.* **9**: 69, 1954.

APPENDIX

Hormone determination

The determination of urinary hormone excretion was carried out by the routine clinical methods used in our laboratory. The technic for total neutral 17-ketosteroids was a modification of that described by Drekter *et al.* (15). Bioassay for estrogens was performed at selected levels in castrated rats, using a modification of the Allen-Doisy vaginal smear technic. Bioassay for gonadotropin was performed, using the method of Loraine and Brown (16). Pregnanediol-complex was estimated by a method described by Keller and his co-workers (17). The 17-hydroxycorticoids were determined by the technic of Forsham *et al.* (18). The method of Dingemanse *et al.* (19) was applied for the chromatographic differentiation of the excreted neutral 17-ketosteroids. Urinary dehydroepiandrosterone was estimated by its absorption spectrum, employing the reagent of Allen *et al.* (20).

Estrogens

Chemical method

Hydrolysis: A 40-ml. aliquot of a 24-hour urine specimen is subjected to hydrolysis with 4 ml. of conc. HCl for thirty minutes under reflux. After the addition of 5 grams of solid NaCl, the mixture is cooled.

Extraction and purification: The hydrolysate is extracted four times with 50 ml. of ether. The ether phases are washed three times with 10 ml. of $NaHCO_3$ (saturated), twice with 10 ml. of Na_2CO_3 (10%) and twice with 10 ml. of water. The combined ether phases are evaporated to dryness at a reduced pressure. The residue is dissolved in 5 ml. of ether and 54 ml. of CCl_4. Extraction is performed with 4×50 ml. of KOH (1*N*). The combined aqueous phases are acidified with diluted H_2SO_4 (Congo blue) under thorough cooling. Extraction is performed with 4×50 ml. of ether. The ether phases are washed twice with 10 ml. of $NaHCO_3$ (saturated) and three times with 10 ml. of water. The ether is evaporated to dryness at reduced pressure.

Chromatography: The residue is chromatographed on 3 grams of alumina (Riedel de Haën), standardized according to Brockmann. The tube diameter is 10 mm. The chromatographic column is prepared in benzene. The column is eluted with 35 ml. of benzene, which is discarded. The estrone-estradiol fraction is then eluted with 35 ml. of chloroform. The estriol fraction is eluted with 35 ml. of methanol. The solvents are evaporated to dryness at reduced pressure.

Fluorometric determination: The 2 fractions (estrone-estradiol, and estriol) are each dissolved in 4 ml. of absolute ethanol. For the fluorometric test, 1 ml. of alcoholic solution from each fraction is mixed with 15 ml. of H_2SO_4 (90%) and the mixture kept at 100° C. for five minutes. After cooling to room temperature, the fluorometric readings are carried out (in our case, with a "Lumetron apparatus"), using an estrone standard solution of 5 I.U. per 1 ml. of absolute ethanol.

The *precision* of our method has been tested by duplicate analyses. The standard deviation between duplicates (in terms of I.U. per twenty-four hours) varies from about 5 to approximately 10 I.U., according to the different excretion levels.

The *sensitivity* of the method is around 10 to 15 I.U. per twenty-four hours.

The average *accuracy* was tested by adding small amounts of estrogens to urine that had been subjected to hydrolysis. The mean recoveries ranged between 82 and 90 per cent for estrone and estradiol, and between 70 and 89 per cent for estriol.

The *normal range* of urinary excretion of estrogens during the normal menstrual cycle is: for the estrone-estradiol fraction, 50 to 450 I.U. per twenty-four hours; and for the estriol fraction, 50 to 500 I.U. per twenty-four hours.

Paper chromatography

For the paper chromatographic determination of urinary estrogens, 50 ml. of a 24-hour specimen of urine was subjected to hydrolysis with 5 ml. of conc. HCl for thirty minutes under reflux. The hydrolysate was extracted and purified in the way described in the preceding section. Instead of column chromatography, paper chromatography was applied, using Whatman No. 1 filter paper. The analytical process is schematically shown in Table 4. It is based on the technics developed by Bush (21) and by Rohr and his co-workers (22). The crude residue was dissolved in benzene and the solution placed on the paper by means of a micrometer syringe. Descending development was employed. The substances were detected by spraying the dried chromatograms with a mixture of equal aqueous volumes of 1 per cent ferric chloride and 1 per cent potassium ferricya-

TABLE 4. SCHEME FOR THE ROUTINE PAPER CHROMATOGRAPHIC FRACTIONATION OF URINARY ESTROGENS

Phenolic fraction

Development in the amido-formic-acid/benzene system.

Area with an R_f like estriol	Area with an R_f like estradiol-17β	Area with an R_f like estrone
Development in the Bush B_5-system	Development in the Bush B_3-system	Development in the Bush B_3-system
Estriol	Estradiol-17β	Estrone

nide, giving a permanent blue-green stain (23). Approximate estimations are possible by visual comparison with spots obtained from known concentrations of the same substance developed on the same sheet of paper.

We found approximately 2.5 micrograms (μg.) of estriol per 50 ml. of urine, and also 2 unidentified phenolsteroids (Fig. 10). Approximately 1 μg. of estrone and approximately 1 μg. of estradiol-17β were also detected per 50 ml. of urine. A photograph of paper chromatogram of the isolated estrone and estradiol fractions cannot be shown, because of non-reproducibility in printing.

Chemical examination of the tumor (540 Gm.)

The tumor was examined chemically in our laboratories. The material was immediately divided into small pieces and homogenized for fifteen minutes in NaCl-solution, with the addition of ice. The extraction technic of Rohr and his co-workers (22) was employed. It is schematically described in Table 5. Descending development was used for the paper chromatographic determination of the various steroids. The chromatographic process for the separation and identification is shown in Tables 4 and 6.

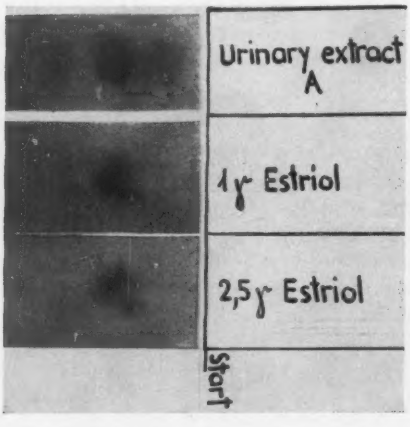

FIG. 10. Paper chromatographic analysis of a urinary extract (from 50 ml. of urine). Photograph of paper chromatogram of the isolated estriol fraction (urinary extract A) with estriol reference standards. The 2 spots on each side of the estriol spot represent unidentified phenolsteroids.

TABLE 5. CHEMICAL ANALYSIS OF HOMOGENIZED TUMOR MATERIAL

Extracted with 2×2,000 ml. of acetone at room temperature.
Extracted with 2×2,000 ml. of acetone under reflux.
Combined extracts evaporated to dryness at reduced pressure. Residue was distributed between petroleum ether and methanol (90, 80, 70 and 50%).

Petroleum-ether phase
Solvent evaporated to dryness at reduced pressure.
Residue (8.149 Gm.) discarded.

Aqueous methanolic phase
Concentrated to 100 ml. Extracted with 5×150 ml. of ethylene chloride. Extracts combined and evaporated to dryness at reduced pressure. Residue (235 mg.) digested with 500 ml. of benzene, filtered, and the solvent evaporated at reduced pressure. Residue distributed between petroleum-ether and 50% aqueous methanol (5×100 ml. of petroleum-ether and 100 ml. of 50% methanol).

Petroleum-ether phase
Extracts combined and evaporated to dryness at reduced pressure. Residue (150 mg.) discarded.

Aqueous methanolic phase
Extracts combined and evaporated to dryness at reduced pressure. Residue (54.4 mg.) dissolved in 50 ml. of benzene. Washed with 2×10 ml. of diluted HCl (0.5 N), 3× with 10 ml. of $NaHCO_3$ (saturated), 5× with 25 ml. of NaOH (1*N*) and 3× with 10 ml. of NaCl-solution (saturated).

$NaHCO_3$ phase
Acidified with diluted H_2SO_4. Extracted with 3×300 ml. of ether-chloroform (3:1). Solvent evaporated to dryness at reduced pressure. Residue (15.9 mg.) discarded.

NaOH phase
Acidified with diluted H_2SO_4. Extracted with 3×300 ml. of ether-chloroform (3:1). Solvent evaporated to dryness at reduced pressure. Residue (5.0 mg.) = *Phenolic fraction*

↓

Paper chromatography (Table 4).

Benzene phase
Evaporated to dryness at reduced pressure. Residue (8.2 mg.) = *Neutral fraction*

↓

Paper chromatography (Table 6).

Solvent systems:

Bush C: toluene-ethyl acetate-methanol-water,
45 : 5 : 25 : 25

Bush B_5: benzene-methanol-water,
10 : 5 : 5

Bush B_3: n-heptane-ligroine-benzene-methanol-water,
1 : 1 : 1 : 2.4 : 0.6

(*Continued on the next page*)

Bush A: n-heptane-methanol-water,
5 : 4 : 1
Amidoformic acid—chloroform system,
Amidoformic acid—benzene system,
Propylene glycol—toluene system,
Propylene glycol—ligroine system.

Cortisone, hydrocortisone and aldosterone were detected by spraying the dried chromatograms with blue tetrazolium solution, yielding blue spots which showed (after heating at 90° C.) a characteristic yellow fluorescence with ultraviolet. Androsterone and dehydroepiandrosterone were detected by spraying the dried chromatogram with a 10 per cent solution of phosphomolybdic acid in ethanol; after heating at 90° C., this produced dark blue spots. Δ^4-Androstene-dione-3,17 shows a characteristic fluorescence with ultraviolet after spraying with a 2*N* solution of NaOH and heating at 90° C. Approximate quantitative estimations were possible by visual comparison with spots obtained from known concentrations of the same substances developed on the same sheet of paper.

TABLE 6. PAPER CHROMATOGRAPHIC DETERMINATION OF THE STEROIDS

Neutral fraction (8.2 mg.)
Development in the Bush C-system.

Start area
Discarded.

Middle area
Eluted. Developed in the amidoformic acid/chloroform system.

Front area
Eluted. Developed in the Bush B_3-system.

Area with an R_f like hydrocortisone. Eluted. Developed in the amidoformic acid/chloroform system.
↓
5 µg. of Hydrocortisone.

Area with R_f like cortisone. Eluted. Developed in the Bush C-system.
↓
2 µg. of Aldosterone.
8 µg. of Cortisone.

Area with an R_f like androsterone and Δ^4-androstene-dione-3,17. Eluted. Developed in the Bush A-system.
↓
10 µg. of Δ^4-Androstene-dione-3,17.
4 µg. of Androsterone.

Area with an R_f like dehydroepiandrosterone. Eluted. Developed in the Bush A-system. Eluted. Developed in the Bush B_3-system.
↓
8 µg. of Dehydroepiandrosterone.

URINARY EXCRETION BY MAN OF 11β,17α,20α, 21-TETRAHYDROXY-4-PREGNEN-3-ONE AND THE 20β EPIMER*

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ABSTRACT

Reduction of the C-20 ketonic group to the 20α hydroxy and 20β epimer appears to be one of the major metabolic pathways of steroid metabolism in man. This study reports the identification of 11β, 17α, 20α,21-tetrahydroxy-4-pregnen-3-one and the 20β epimer isolated from extracts of the urine 1) from a man receiving adrenocorticotropin, 2) from a woman with hypopituitarism, after she had ingested 400 mg. of cortisol acetate, 3) from 3 "normal" men (a "pool"), and 4) from a normal man. These results confirm the findings of Peterson, Pierce and Kliman after administration of cortisol to normal men, and of Holness, Lunnon and Gray who found the 20β epimer after the administration of adrenocorticotropin. In addition this study reports the findings of the 20α epimers after administration of adrenocorticotropin, and both epimers in urine from normal men.

INTRODUCTION

THE urinary excretion of 11β,17α,20α,21-tetrahydroxy-4-pregnen-3-one and its 20β epimer following administration of cortisol to "normal subjects" has been reported by Peterson, Pierce and Kliman (1, 2). Holness, Lunnon and Gray have reported isolation of the 20β epimer from the urine of a patient with lupus erythematosus receiving 40 mg. of adrenocorticotropin (3). Our observations confirm these findings, and in addition we report the isolation of both these substances from the urine of normal men and of the 20α as well as the 20β epimer after administration of adrenocorticotropin.

SUBJECTS AND METHODS

Subjects

Sixteen liters of urine were collected during their working hours from 3 normal men. This collection is hereafter called "normal pool."

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Subject TOO., a "normal" man except for keratoconus, collected urine for three days starting the fifth day after a "corneal transplant" operation.

Subject AL. was a woman with severe hypopituitarism. Urine excreted during the 24-hour period following ingestion of 400 mg. of cortisol acetate was used for this analysis. The alpha-ketolic steroids isolated from this urine, and the clinical status of the patient have been described previously (4).

Subject DOW., with multiple sclerosis and hypertension, was given 20 units of adrenocorticotropin intravenously over eight hours each day during a ten-day collection period.

Chemical methods

The basic methods that were employed have been previously described (4). The glucuronidase hydrolytic procedure for urine from subjects other than AL. was modified. It was found that a greater yield of alpha-ketolic steroids was achieved by glucuronidase hydrolysis for forty-eight hours in the presence of a half volume of chloroform which was constantly stirred. The urine was then separated and extracted at pH 4.5 and then at pH 1 with chloroform for one hour. The following chromatographic systems were employed: 1) toluene:propylene-glycol, 2) chloroform:formamide, 3) benzene:formamide, and 4) methylcyclohexane:propylene-glycol.

Most of the identification techniques have been reported previously in some detail (4). In general, they are adapted from those described by Zaffaroni (5). In addition, Cifonelli and Smith's test for oxygen on adjacent carbon atoms was slightly modified for use with steroids (6). It was found that the presence of 5 micrograms (μg). of steroids with adjacent hydroxyl groups could be detected by this method if "spotted" on the paper.

Infrared spectrometric analysis was performed through the courtesy of Dr. Thomas Gallagher at the Sloan-Kettering Institute for Cancer Research, Memorial Center, New York. The reference steroids were kindly furnished by Dr. Durey H. Peterson, The Upjohn Company, Kalamazoo, Michigan.

RESULTS

The urinary extracts were subjected to preliminary chromatography in the toluene:propylene-glycol system for ninety hours. Re-chromatography of the eluates of the tetrahydrocortisol region in the same system for 162 hours, or in chloroform:formamide for forty-two to forty-five hours separated the two epimers, as shown in Figures 1 and 2. The epimers were acetylated and re-run in toluene:propylene-glycol for seven hours or methylcyclohexane: propylene-glycol for fourteen days. The materials obtained were submitted to other chromatographic and functional group tests as shown in Tables 1 and 2. The diacetates (Subject DOW., extract and standards) were subjected to chromatography on celite-silica gel prior to submission for infrared spectrometry. Infrared spectrometry was performed by Beatrice S. Gallagher at the Sloan-Kettering Institute for Cancer Research, Memorial Center, New York City. The report from Dr. Thomas Gallagher is as follows: "The spectra of the diacetates were examined between 850 and 1200 cm.$^{-1}$ in chloroform solution using a Perkin-Elmer model 12-C infrared spectrophotometer. The spectra were com-

TABLE 1. ISOLATION OF 11β, 17α, 20α, 21-TETRAHYDROXY-4-PREGNEN-3-ONE FROM URINE EXTRACTS

Procedure*	Standard	Subject DOW. (mult. sclerosis) ACTH	Subject AL. (hypopit.) 400 mg. cortisol	3 Normal men (pooled urine)	Subject TOO. (normal) man
R_{DOC} in toluene:propylene-glycol system	.0048	.0047	.0040	.0040	.0047
R_E in $CHCl_3$:formamide	.16	.16	.16	.16	.17
R_E in benzene:formamide	.16	.15	—	.14	.14
R_{DOC} of diacetate in methyl-cyclohexane: propylene-glycol	.019	.020	.020	.020	.020
R_{DOC} of diacetate in toluene:propylene-glycol	.30	.31	.30	.30	.27
R_{DOC} of CrO_3 oxidation product of diacetate in methylcyclohexane:propylene-glycol	.10†	.11†	—	—	.10†
R_{DOC} of sodium bismuthate oxidation product	.50‡	.52‡	—	—	.47‡
Fluorescence in H_2SO_4	++	++	++	++	++
Spectrometry in H_2SO_4	+	+	+	+	+
Soda fluorescence test	+	+	+	+	+
Absorption maxima in ethanol	240	238	238	240	242
Reaction with blue tetrazolium	0	0	0	0	0
KIO_4-benzidine test (modified, Cifonelli and Smith)	+	+	+	+	+
Infrared spectrometry	+	+	—	—	—

* R_{DOC} = Running rate related to 11-desoxycorticosterone.
R_E = Running rate related to cortisone.

† R_{DOC} of 20, 21-diacetate of 17α, 20α, 21-trihydroxy-4-pregnene-3,11-dione = .10.

‡ Positive results with Zimmermann test. R_{DOC} of 11β-hydroxy-4-androstene-3,17-dione = .50.

pared with those of authentic reference compounds. All major absorption bands were coincident in position and the relative intensities were similar. There was a small band at 870 cm.$^{-1}$ attributed to slight impurity."

The absorption spectra in sulfuric acid for the 20α and 20β epimers isolated from the urine of Subject DOW. after administration of adrenocorticotropin are presented in Figures 3 and 4. The absorption spectra in sulfuric acid appeared to be unique, as judged by the reports of Zaffaroni (5), Bernstein and Lenhart (7) and our own experience.

The quantitative aspects of the fluorescence in sulfuric acid calls for comment. This was determined by a modification (4) of the method of Engel *et al.* (8) for estrogens. Two-tenths of a milliliter of a solution of

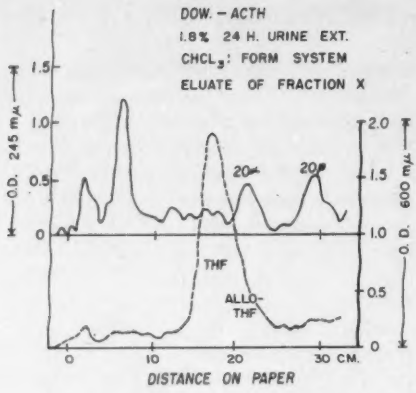

FIG. 1. Separation of 11β,17α,20α,21-tetrahydroxy-4-pregnen-3-one and the 20β epimer in the chloroform:formamide system. Urine extract, subject DOW. The steroids in the region more polar than cortisol were eluted from chromatograms run in the toluene:propylene-glycol system and then re-run in the chloroform:formamide system for 43 hours. The extract on this chromatogram represents 1.8 per cent of a 24-hour aliquot of urine collected for ten days during treatment with injections of adrenocorticotropin. The tetrahydrocortisol (THF) and allotetrahydrocortisol (allo-THF) positions are indicated.

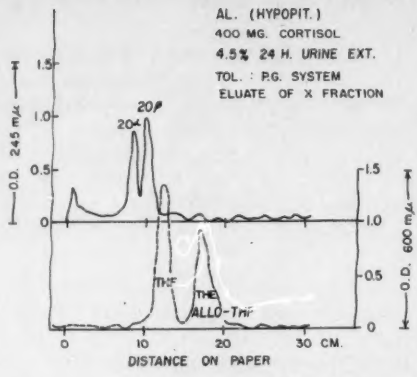

FIG. 2. Separation of 11β,17α,20α,21-tetrahydroxy-4-pregnen-3-one and the 20β epimer in the toluene:propylene-glycol system. Urine extract, Subject AL. The steroids more polar than cortisol were eluted from the preliminary chromatograms run in the toluene:propylene-glycol system. These were re-run for 162 hours. The paper was saturated with propylene-glycol:methanol (1:1) prior to application of the eluates. The extract on this chromatogram represents 4.5 per cent of the urine collected for 24 hours after ingestion of 400 mg. of cortisol acetate. The tetrahydrocortisol (THF), tetrahydrocortisone (THE) and allo-tetrahydrocortisol (Allo-THF) positions are indicated.

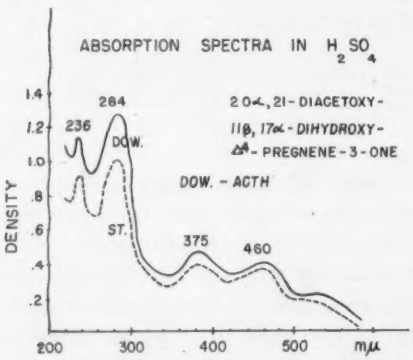

FIG. 3. Absorption spectrum in sulfuric acid of 20α,21-diacetoxy-11β,17α-dihydroxy-4-pregnen-3-one isolated from the urine of Subject DOW., and that of the standard (St.). Also see Table 1, methods and results.

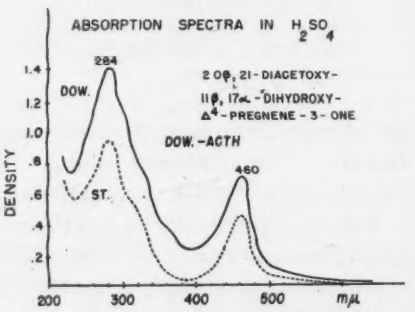

FIG. 4. Absorption spectrum in sulfuric acid of 20β,21-diacetoxy-11β,17α-dihydroxy-4-pregnen-3-one isolated from the urine of Subject DOW., and that of the standard (St.). Also see Table 2, methods and results.

TABLE 2. ISOLATION OF 11β,17α,20β,21TRETRAHYDROXY-4-PREGNEN-3-ONE FROM URINE EXTRACTS

Procedure*	Standard	Subject DOW. (mult. sclerosis) ACTH	Subject AL. (hypopit.) 400 mg. cortisol	3 Normal men (pooled urine)	Subject TOO. (normal) man
R_{DOC} *in toluene:propylene-glycol system*	.0064	.0066	.0048	.0060	.0060
R_E in $CHCl_3$:formamide	.22	.22	.21	.21	.22
R_E in benzene:formamide	.18	.18	—	.15	.17
R_{DOC} of diacetate in methylcyclohexane: propylene-glycol	.016	.015	.015	.014	.014
R_{DOC} of diacetate in toluene:propylene-glycol	.18	.17	.17	.17	.18
R_{DOC} of CrO_3 oxidation product of diacetate in methylcyclohexane:propylene-glycol	.070†	.072†	—	—	.060†
R_{DOC} of sodium bismuthate oxidation product	.50‡	.46‡	—	.40‡	.47‡
Fluorescence in H_2SO_4	+	+	+	+	+
Spectrometry in H_2SO_4	+	+	+	+	+
Soda fluorescence test	+	+	+	+	+
Absorption maxima in ethanol	242	242	240	240	240
Reaction in blue tetrazolium	0	0	0	0	0
KIO_4-benzidine test (modified, Cifonelli and Smith	+	+	+	+	+
Infrared spectrometry	+	+	—	—	—

* R_{DOC} = Running rate related to 11-desoxycorticosterone.
R_E = Running rate related to cortisone.
The running rates listed in Tables 1 and 2 are those obtained after purification in one or more solvent systems, with the exception of the rates of the free compounds in the toluene:propylene-glycol system for epimers in the extract of urine from Subject AL. The slower rates in this case are due to a displacement effect. This was demonstrated by running a mixture of the standard 20α and 20β epimers plus authentic tetrahydrocortisol and tetrahydrocortisone in the toluene:propylene-glycol system. The mobilities (R_{DOC}) of the epimers under these circumstances were .0030 and .0040 respectively.
† R_{DOC} of 20, 21 diacetate of 17α, 20β, 21-trihydroxy-4-pregnene-3,11-dione = .070.
‡ Positive results with Zimmermann test. R_{DOC} of 11β-hydroxy-4-androstene-3,17-dione = .50.

of ethanol:toluene (1:19) was added to 4 to 25 μg. of the steroid. After addition of 1 ml. of 86.5 per cent H_2SO_4 (w/w), the tube was shaken and allowed to stand at room temperature for fifteen minutes. Then 9 ml. of 64.5 per cent H_2SO_4 (w/w) was added and the fluorescence was measured in a Lumetron fluorometer using the "B_2" primary and secondary filters.

Both the chromatographically purified reference standard of 11β,17α,20α, 21-tetrahydroxy-4-pregnen-3-one and the purified 20α epimer from the urine of Subject DOW. exhibited fluorescence (on a weight basis) at least equal to that of corticosterone. The purified standard 20β epimer and that from the urine of Subject DOW. possessed about 7 per cent of the fluorescence of the 20α epimer. This is in contrast to the results of Peterson *et al.* (1), who found by a different technique that both the 20α and 20β epimers exhibited approximately 50 per cent of the fluorescence produced by corticosterone. The 20α and 20β epimers isolated from the urine of Subjects AL. and TOO. and from the "normal pool" showed essentially the same degree of fluorescence as the standard compounds.

The unknowns isolated from the urine of Subjects AL. (hypopituitarism) and TOO. (normal) and from the "normal pool" were submitted to a variety of chromatographic and microanalytic tests shown in Tables 1 and 2. The amounts of these unknowns were insufficient for analysis by infrared spectrometry. However, the concordance of all characteristics with the reference standards substantiates their identities. The strong fluorescence in sulfuric acid of the 20α epimer and the excellent agreement of the characteristic absorption curves in sulfuric acid with those of the reference standards are particularly important in this respect.

The approximate amount of Δ^4-3-ketonic steroids, with maximum absorption at 240 mμ, on the paper chromatogram may be crudely estimated by measuring the area under the curve of the density against distance (Fig. 2). A wave length of 245 mμ is used, since the absorption by the paper rises sharply below this point. This method is subject to considerable error, particularly if a large amount of the steroid is present. In general, most estimates are within $\pm$30 per cent. With these considerations in mind, the following may be regarded as approximations of the amounts of 11β,17α,20α,21-tetrahydroxy-4-pregnen-3-one and the 20β epimer extracted per 24-hour aliquot of urine:

	20α Form	20β Epimer
	(mg./24 hrs.)	(mg./24 hrs.)
Subject DOW. (ACTH)	0.4	0.6
Subject AL. (400 mg. cortisol)	0.5	0.6
Three "Pool" normal men	0.04	0.04
Subject TOO., normal man	0.2	0.15

DISCUSSION

Various metabolites of the adrenocortical steroids with a hydroxyl group at carbon atom 20 have been isolated from human urine. However,

only 4 steroids with hydroxyls at carbon atom 20 and 21 and a Δ^4-3-ketone in ring A have been reported as isolated from this source. Peterson found 11β,17α,20α,21-tetrahydroxy-4-pregnen-3-one and its 20β epimer following the administration of 200 mg. of cortisol per day (1, 2). The 20β epimer has been isolated from the urine of a subject receiving adenocorticotropin by Holness, Lunnon and Gray (3). In addition, the latter authors isolated 17α,20β,21-trihydroxy-4-pregnene-3,11-dione. Lombardo and Hudson reported the identification of this steroid following the administration of 765 mg. of cortisone acetate during a period of twenty-six days to an adrenalectomized man (9). We have recently identified 11β,20β,21-trihydroxy-4-pregnen-3-one isolated from the urine extract in the case of hypopituitary Subject AL. after the administration of 200 mg. of 11-dehydrocorticosterone acetate per day for two days (10).

Reduction of the ketone group of carbon atom 20 of steroids not having a 21-hydroxyl group and reduced in ring A (*e.g.*, pregnandiol) has been well known (11) for many years. Fukushima and his co-workers in 1955 (12) reported the isolation from human urine of 4 metabolites of cortisol reduced in ring A and at the C-20 position (cortol, β-cortol, cortolone, and β-cortolone). These 4 compounds represented a minimum of 30 per cent of the metabolites of cortisol-4-C^{14} present in the urine extract. It thus appears that in the human, as well as in other species, reduction of the ketone at C-20 is one of the major metabolic pathways not only of methyl ketones, such as progesterone, but also of alpha-ketolic steroids of the cortisol and corticosterone type.

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REFERENCES

1. Peterson, R. E.; Pierce, C. E., and Kliman, B.: Isolation of Δ^4-pregnen -11β, 17α, 20α, 21-tetrol-3-one in the urine of man, *Arch. Biochem. & Biophys.* **70:** 614, 1957.
2. Peterson, R. E.; Wyngaarden, J. B.; Guerra, S. L.; Brodie, B. B., and Bunim, J. J.: The physiological disposition and metabolic fate of hydrocortisone in man, *J. Clin. Invest.* **34:** 1779, 1955.
3. Holness, N. J.; Lunnon, J. B., and Gray, C. H.: The identification of some adrenocortical steroids in urine, *J. Endocrinol.* (British) **14:** 138, 1956.
4. Richardson, E. M.; Touchstone, J. C., and Dohan, F. C.: Urinary alpha-ketolic steroid metabolites of cortical hormones administered to subjects with adrenal cortical insufficiency, *J. Clin. Invest.* **34:** 285, 1955.
5. Zaffaroni, A.: Micromethods for the analysis of adrenocortical steroids, *in* Recent Progress in Hormone Research, ed. by G. Pincus. New York, Academic Press Inc., 1953, vol. 8, p. 51.

6. CIFONELLI, J. A., and SMITH, F.: Detection of glycosides and other carbohydrate compounds on paper chromatograms, *Analyt. Chem.* **26**: 1132, 1954.
7. BERNSTEIN, S., and LENHART, R. H.: The absorption spectra of steroids in concentrated sulfuric acid. I. Methods and data, *J. Org. Chem.*, **18**: 1146, 1953.
8. ENGEL, L. L.; SLAUNWHITE, W. R., JR.; CARTER, P., and NATHANSON, I. T.: The separation of natural estrogens by counter-current distribution, *J. Biol. Chem.*, **185**: 255, 1950.
9. LOMBARDO, M. E., and HUDSON, P. B.: Urinary steroid excretion after total adrenalectomy. III. The isolation and identification of metabolites of cortisone acetate, *J. Biol. Chem.* **229**: 181, 1957.
10. RICHARDSON, E. M.; BULASCHENKO, H., and DOHAN, F. C.: Identification of Δ^4-pregnene-11β,20β,21-triol-3-one in urine extract after administration of 11-dehydrocorticosterone acetate, *J. Clin. Endocrinol. & Metab.* **18**: 666, 1958.
11. DORFMAN, R. E., and UNGAR, F.: Metabolism of Steroid Hormones. Minneapolis, Burgess Publishing Company, 1953, p. 17.
12. FUKUSHIMA, D. K.; LEEDS, N. S.; BRADLOW, H. L.; KRITCHEVSKY, T. H.; STOKEM, M. B., and GALLAGHER, T. F.: The characterization of four new metabolites of adrenocortical hormones, *J. Biol. Chem.* **212**: 449, 1955.

FRACTIONATED PLASMA PROTEIN VALUES IN SUBACUTE THYROIDITIS

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ABSTRACT

Tiselius fractionation of plasma proteins was performed on 27 patients with subacute (granulomatous) thyroiditis. In 21 there was a significant elevation of the α_2-globulin level and in 18 of these 21 there was a significant elevation of the fibrinogen level.

Other findings of the fractionation of plasma proteins in subacute thyroiditis were: a decrease in the level of plasma albumin in 16 patients, and an elevation in the level of α_1-globulin in 5, of beta-globulin in 2, of gamma-globulin in 12, and of fibrinogen in 19. In only 6 of 55 patients with Hashimoto's disease, and in none of 30 patients with nontoxic adenomatous goiter were high values obtained for α_2-globulin. In 6 of 35 patients with struma lymphomatosa the fibrinogen values were elevated.

The erythrocyte sedimentation rate was increased in 18 of 20 patients in whom it was determined. The rate was elevated in 15 of 16 patients who had a high level of α_2-globulin.

The increase in the level of α_2-globulin probably is largely due to the catabolic effect of the inflammation on thyroidal and, especially, on extrathyroidal tissues. This elevation was less likely to occur in patients who were less febrile, especially in those in whom only one lobe of the thyroid was affected. The diagnostic value of this test is largely limited to those patients in whom the clinical picture is more typical. Furthermore, an elevated level of plasma α_2-globulin is a nonspecific finding, occurring in a number of other diseases, including other inflammations. It is our belief that a high value for α_2-globulin, like the increased erythrocyte sedimentation rate and low thyroidal radioiodine uptake, is a useful finding only when correlated with the clinical picture.

The diagnosis of subacute thyroiditis still is best made on a clinical basis, *i.e.*, history, physical findings, response to prednisone or cortisone, and eventual disappearance of the goiter.

INTRODUCTION

RECENTLY Stemmermann (1) reported the occurrence of an increased concentration of α_2-globulin in the serum of 4 patients with subacute (granulomatous) thyroiditis, as determined by paper electrophoresis. Prior to that observation, 2 cases in which both alpha- and gam-

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ma-globulin values were increased had been described by Fromm and associates (2), and 1 case by Werner (3). Skillern, Nelson and Crile (4) recently reported a study of 6 patients with atypical subacute thyroiditis; in only 1 was there a significant increase in the α_2-globulin level, and in only 2 was there an increase in the gamma-globulin level. The buffer used for Tiselius electrophoresis was phosphate (pH 7.8) in 5 of the patients, and barbital (pH 8.6) in 1. Weissman and Perlmutter (5) in a study of 8 patients with subacute thyroiditis observed consistent elevation in the levels of serum alpha$_1$-, alpha$_2$-, and gamma-globulin. Because of the paucity of cases in the literature, we are presenting our results of fractionation of plasma proteins (using barbital as a buffer) in 27 patients having subacute thyroiditis, in order better to determine its true worth as a laboratory diagnostic aid.

METHOD

Twenty-seven patients, 20 women and 7 men, with subacute thyroiditis comprised the series. In 18 cases the disease involved both thyroid lobes, and in 9 it involved only one lobe. None of the 27 patients had received cortisone or x-ray treatment prior to our determinations. The diagnosis was based on a characteristic history and clinical findings, as well as the results of laboratory tests. A further diagnostic aid was rapid marked amelioration of thyroidal pain and eventual disappearance of the goiter in all patients receiving prednisone.

The blood was drawn from the patients when they first were examined. The plasma proteins were fractionated by Tiselius electrophoresis, using the Longsworth modification (6). A barbital buffer, pH 8.6, ionic strength (μ) 0.10, was used because it permits much more adequate separation of α_1-globulin and α_2-globulin from albumin than can be obtained using a phosphate buffer. Erythrocyte sedimentation rates were determined in 20 of 27 patients according to the Rourke-Ernstene method (7).

RESULTS

In 21 of the 27 patients with subacute thyroiditis there was an elevation of the plasma α_2-globulin level (Table 1). The plasma albumin content was decreased ($<$3.0 Gm. per 100 ml.) in 16 patients. The level of α_1-globulin was increased in 5 patients, of beta-globulin in 2, and of gamma-globulin in 12. The fibrinogen values were significantly increased in 19 of 27 patients tested.

Erythrocyte sedimentation rates were increased in 18 of 20 patients. This determination was carried out in 5 of 6 patients in whom there had been no increase in the level of α_2-globulin; in 3 there was an increased rate (Table 1). In 5 of these 6 patients, the fibrinogen value was not significantly elevated.

In 5 of the 6 patients with no significant elevation of the α_2-globulin level, only one lobe of the thyroid was involved at the time the test was made. In the other 4 patients with involvement of only one lobe, the con-

TABLE 1. RESULTS OF TISELIUS ELECTROPHORETIC FRACTIONATION OF THE PLASMA PROTEINS (BARBITAL BUFFER pH 8.6, μ 0.10) IN 27 PATIENTS, AND THE CORRESPONDING ERYTHROCYTE SEDIMENTATION RATE IN 20 PATIENTS WITH SUBACUTE THYROIDITIS*

Patient No.	Albumin (normal, 3.91 ± 0.57)*	Globulin (gm./100 ml. plasma)				Fibrinogen (normal, 0.44 ± 0.17)	Erythrocyte sed. rate, (normal <0.65 mm./min.)
		α_1 (normal, 0.43 ± 0.10)	α_2 (normal, 0.62 ± 0.12)	β (normal, 0.94 ± 0.14)	γ (normal, 0.75 ± 0.13)		
1†	3.07	0.62	0.92	0.86	1.03	0.75	0.9
2	2.65	0.51	1.28	1.15	1.69	1.15	1.75
3	2.26	0.56	1.22	0.93	0.70	1.05	—
4	3.21	0.54	1.22	1.40	0.78	1.10	—
5	3.54	0.51	1.07	1.07	0.89	0.82	—
6†	2.72	0.65	1.14	1.02	0.59	0.78	1.65
7	2.24	0.60	1.03	0.75	1.74	0.79	2.05
8†	2.96	0.52	0.85	0.87	1.29	0.61	1.0
9	2.96	0.80	0.87	0.82	1.01	0.72	—
10	3.86	0.58	1.03	1.10	1.29	0.89	0.9
11	2.75	0.59	0.91	0.78	1.04	0.91	1.95
12	2.96	0.66	1.15	1.15	0.99	0.99	1.05
13	3.77	0.43	0.93	0.95	0.54	0.35	—
14	2.98	0.49	0.89	0.72	1.34	0.86	0.5
15†	2.37	0.52	0.84	0.95	0.82	1.10	1.15
16	2.86	0.48	0.91	0.78	1.28	0.94	1.5
17	2.41	0.43	0.81	0.77	0.84	0.59	0.75
18	2.79	0.51	0.88	0.82	0.73	0.96	1.6
19	2.76	0.52	0.80	0.96	0.72	0.99	1.25
20	1.73	0.66	1.36	0.59	0.76	0.90	1.45
21	3.03	0.51	0.92	1.04	1.08	0.92	1.45
22†	3.05	0.28	0.65	0.93	0.59	0.55	1.05
23†	3.82	0.60	0.76	0.89	0.64	0.64	0.85
24	3.08	0.44	0.76	0.76	0.46	0.45	—
25†	2.87	0.59	0.30	1.00	1.03	0.81	1.95
26†	3.07	0.51	0.75	0.85	0.83	0.48	0.65
27†	4.43	0.33	0.40	0.63	1.04	0.47	—

* Mean ± S.D. (62 normal subjects).

† Unilobar thyroid involvement when first examined.

centration of α_2-globulin was elevated. At the time the blood was drawn, 3 of these 4 patients were more febrile than the other 6 in the group with only unilobar involvement.

DISCUSSION

Our experience indicates that the diagnosis of subacute (granulomatous) thyroiditis still is best made on the basis of clinical history, physical findings, response to prednisone or cortisone and eventual disappearance of the goiter. Skillern, Nelson and Crile (4) observed asymptomatic goiter in 2 patients with subacute thyroiditis. Woolner and associates (8) later reported an incidence of 33 per cent asymptomatic goiter in their series of 108 patients. In a series of 50 patients seen by one of us (P.G.S.) only 2 had no history of symptomatic or painful goiter at any time. In these 2 patients the diagnosis was proved by needle biopsy. We have seen a number of patients who had goiters that were painless on first examination,

but careful questioning elicited a past history of subjective pain or tenderness to palpation and often other febrile symptoms.

Laboratory tests at best can only be regarded as useful aids and the results must be correlated with the clinical picture. A low thyroidal radioiodine uptake and an increased erythrocyte sedimentation rate furnish important information, but we have found normal values for these determinations in some patients with subacute thyroiditis. Likewise, an elevated level of plasma α_2-globulin may be a helpful finding—but only when correlated with the clinical picture. The lack of significant elevation of the α_2-globulin level in 5 of the 9 patients with involvement of only one thyroid lobe, or in whom the reaction to the thyroiditis was less febrile at the time blood was drawn, decreases the value of this test as a diagnostic aid. Thus, our results do not confirm the consistency of the elevation of α_2-globulin concentration in subacute thyroiditis reported by Stemmermann (1) and by Weissman and Perlmutter (5). The lack of increase in the plasma α_2-globulin level in 5 of 9 cases of subacute thyroiditis involving only one lobe of the thyroid may have been due to the small amount of tissue affected and the decreased febrile reaction; this may also have been the explanation in the less febrile patients in whom both lobes of the thyroid were affected. Skillern, Nelson and Crile (4) reported a patient with a diffuse hard asymptomatic goiter, who had no fever or pain at any time during the course of the disease, but had marked anemia and a normal level of plasma α_2-globulin. The diagnosis of subacute thyroiditis was proved by needle-biopsy findings.

The age and sex of the patient and the estimated duration of the disease prior to the first examination did not appear to be factors in determining the presence of elevated levels of α_2-globulin and fibrinogen. The duration prior to examination varied from one to twelve weeks (average, five weeks), although the total course of the disease was longer.

The finding of elevated α_2-globulin values in 6 of 55 patients with struma lymphomatosa, somewhat decreased the usefulness of this test in differential diagnosis. This plasma protein fraction was not elevated in 30 patients with nontoxic adenomatous goiter. The gamma-globulin level was increased in 42 of 55 patients with struma lymphomatosa, and in 12 of 27 patients with subacute thyroiditis. Thus, the level of gamma-globulin, in itself, is of little value as an aid in differentiation. The cause of the elevation of gamma-globulin concentration in 12 of 27 patients is unknown. It is doubtful that it is accounted for solely by antibodies to thyroglobulin, since a search for the presence of such antibodies in 2 of our patients with elevated gamma-globulin values had negative results. Of 35 patients with struma lymphomatosa who were tested, 6 had a significantly elevated

level of plasma fibrinogen. Five of these 6 patients had clinical hypothyroidism.

It should be pointed out that all of the changes in plasma protein concentration other than that in fibrinogen can be estimated by paper electrophoresis as well as by the Tiselius procedure.

Stemmermann (1) suggested that the increased concentration of plasma α_2-globulin in cases of subacute thyroiditis is caused by the escape of thyroglobulin-containing colloid into the blood stream as a result of destruction of the follicles of the thyroid gland. Although destruction of the thyroid cells and release of the hormone may play a small part, it is doubtful that the small amount of thyroglobulin involved would be sufficient to cause elevation of the plasma α_2-globulin level. We believe that the increased concentration of α_2-globulin probably is caused for the most part by destruction and release of protein from extrathyroidal body tissue consequent to the thyroidal inflammation. Weissman and Perlmutter (5) have expressed the belief that this elevation of α_2-globulin concentration in subacute thyroiditis is due to the effects of the inflammation on extrathyroidal tissues. Longsworth and associates (10) noted increased α_2-globulin levels in patients having other febrile conditions. Shedlovsky and Scudder (11) showed the presence of elevated α_2-globulin levels in various other febrile conditions involving inflammation or tissue destruction. This work has been substantiated by others (12–14). In addition to the catabolic effects of inflammation, another factor that may cause elevation of the α_2-globulin concentration in subacute thyroiditis is the liberation of excessive amounts of thyroxine into the plasma because of destruction of the thyroid cells. Lewis and McCullagh (9) reported that the plasma α_2-globulin level is elevated in a significant percentage of patients with hyperthyroidism due to Graves' disease. This elevation may be due in part to catabolism induced by action of an excessive amount of thyroxine on extrathyroidal body tissue.

The occurrence of 18 elevated fibrinogen values in 21 patients with elevated α_2-globulin values, and of normal fibrinogen values in 5 of 6 patients with normal α_2-globulin values, suggests a correlation between α_2-globulin and fibrinogen. The cause of the high plasma fibrinogen values is not known. Shedlovsky and Scudder (11), Gray and Mitchell (12), and Malmros and Blix (13) have pointed out the correlation of elevated plasma α_2-globulin and fibrinogen levels in other diseased states. They (11–13) also have pointed out that changes in these 2 protein fractions largely account for the increased erythrocyte sedimentation rate. Gray and Mitchell (12) reported that an elevation in the plasma fibrinogen level increased the erythrocyte sedimentation rate more effectively than did elevations of

other protein fractions; alpha and beta fractions were next most effective, and gamma-globulin was the least effective. They also stated that a low concentration of plasma albumin is a factor in increasing the sedimentation rate.

REFERENCES

1. Stemmermann, G. N.: Serum protein changes in subacute thyroiditis, *J.A.M.A.* **162:** 31 (Sept. 1) 1956.
2. Fromm, G. A.; Lascano, E. F.; Bur, G. E., and Escalante, D.: Tiroiditis crónica inespecifica; estruma linfoideo (Hashimoto). Estruma granulomatosa (de Quervain). Estruma fibroso (Riedel), *Rev. Asoc. méd. argent.* **67:** 162 (May 15–30) 1953.
3. Werner, S. C. (Editor): The Thyroid: A Fundamental and Clinical Text. New York, Paul B. Hoeber, Inc., 1955, p. 747.
4. Skillern, P. G.; Nelson, H. E., and Crile, G., Jr.: Some new observations on subacute thyroiditis, *J. Clin. Endocrinol. & Metab.* **16:** 1422 (Nov.) 1956.
5. Weissman, N., and Perlmutter, M.: An electrophoretic study of serum proteins in thyroiditis, *J. Clin. Invest.* **36:** 780 (June) 1957.
6. Longsworth, L. G.: Modification of schlieren method for use in electrophoretic analysis, *J. Am. Chem. Soc.* **61:** 529 (Feb.) 1939.
7. Rourke, M. D., and Ernstene, A. C.: A method for correcting the erythrocyte sedimentation rate for variations in the cell volume percentage of blood, *J. Clin. Invest.* **8:** 545 (June) 1930.
8. Woolner, L. B.; McConahey, W. M., and Beahrs, O. H.: Granulomatous thyroiditis (de Quervain's thyroiditis), *J. Clin. Endocrinol. & Metab.* **17:** 1202 (Oct.) 1957.
9. Lewis, L. A., and McCullagh, E. P.: Electrophoretic analysis of plasma proteins in hyperthyroidism and hypothyroidism, *Am. J. M. Sc.* **208:** 727 (Dec.) 1944.
10. Longsworth, L. G.; Shedlovsky, T., and MacInnes, D. A.: Electrophoretic patterns of normal and pathological human blood serum and plasma, *J. Exper. Med.* **70:** 399 (Oct.) 1939.
11. Shedlovsky, T., and Scudder, J.: A comparison of erythrocyte sedimentation rates and electrophoretic patterns of normal and pathological human blood, *J. Exper. Med.* **75:** 119 (Jan.) 1942.
12. Gray, S. J., and Mitchell, E. B.: Effect of purified protein fractions on sedimentation rate of erythrocytes, *Proc. Soc. Exper. Biol. & Med.* **51:** 403 (Dec.) 1942.
13. Malmros, H., and Blix, G.: The plasma proteins in cases with high erythrocyte sedimentation rate, *Acta med. scandinav.* Suppl. 170, pp. 280–306, 1946.
14. Lewis, L. A.: Electrophoresis in Physiology. American Lectures in Medical Physics, No. 89. Springfield, Illinois, Charles C Thomas, Publisher, 1951.

SIMPLIFIED WATER LOADING TEST IN HYPOADRENOCORTICISM AND HYPOTHYROIDISM*

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ABSTRACT

Soffer and Gabrilove introduced a simplified water loading test in 1952 to aid in the diagnosis of adrenocortical insufficiency. The present data confirm these findings in 33 patients with primary (Addisonian) or secondary (hypopituitary) hypoadrenocorticism. Thirteen of the 18 patients with hypopituitarism had unequivocal hypothyroidism. After water loading, all patients tested had impaired diuretic responses which showed substantial improvement when the tests were repeated after administration of cortisone. In contrast, the 9 patients with primary hypothyroidism responded normally to water loading. Hypothyroidism associated with an impaired water tolerance which improves distinctly during administration of cortisone, is probably secondary to pituitary failure. Hypothyroidism associated with normal water tolerance is suggestive of primary thyroidal dysfunction.

THIS study represents an extension of the findings of Soffer and Gabrilove in regard to the usefulness of the simplified water loading test in the diagnosis of adrenocortical insufficiency. In addition it demonstrates the value of the procedure in distinguishing primary from pituitary myxedema.

Impaired water diuresis following water loading has been observed in hypoadrenocortical states for many years. Rosenow noted in 1925 that in patients with Addison's disease no diuresis occurred after a large intake of water (1). Rowntree and Snell in 1931 and Vitoria in 1935 made similar observations (2, 3). In 1941 Robinson, Power and Kepler first applied these observations clinically in the diagnosis of adrenal insufficiency (4). More recently it has been noted that the diuretic response to water loading reverted to normal in patients with primary adrenal insufficiency given cortisone and in patients with pituitary insufficiency given cortisone or corticotropin (5–7). Soffer and Gabrilove modified and simplified the Robinson, Power and Kepler procedure and sharpened the specificity by

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repeating the test after the administration of cortisone when the initial diuresis was impaired (8).

In regard to primary myxedema, Crispell and his associates reported an abnormal diuretic response following the ingestion of a water load in patients with longstanding primary thyroidal insufficiency (9). The abnormality was not corrected with cortisone but there was a reversion to normal with correction of the hypothyroid state. However, Gaunt had demonstrated that rats made hypothyroid by the administration of goitrogens for three to eight weeks, or by surgery, responded normally to water loading (10, 11).

METHODS

The water loading test that we employed consists of the oral administration of 1500 ml. of tap water[1] over a period of fifteen to forty-five minutes to patients who have been fasting overnight. The five-hour urine specimen collected from the beginning of the test is measured. During the five-hour period the patients recline or sit, except while voiding. A urine volume of less than 800 ml. is considered abnormal.

RESULTS

The results of the water loading tests in 3 groups of cases—Addison's disease, hypopituitarism and primary hypothyroidism—are shown in Figure 1.

Fifteen patients with Addison's disease were studied both before (untreated) and during administration of desoxycorticosterone. In all, the initial five-hour urine volumes were reduced, the largest volume being 630 ml. Tests repeated two hours after the oral administration of 50 mg. of cortisone showed a considerable increase in the five-hour volumes.

Eighteen patients with hypopituitarism were similarly studied. Of this group, 13 had unequivocal hypothyroidism, 1 was euthyroid, and in the remaining 4 patients the thyroid status was not definitely ascertained. Both before and after cortisone therapy, the findings in the 18 patients were similar to those in the patients with Addison's disease. The urine volumes prior to the administration of cortisone varied from 55 to 765 ml. Tests repeated during cortisone therapy showed a substantial increase in the five-hour volume of urine although, as in the patients with Addison's disease, the post-cortisone five-hour volumes were not always greater than 800 ml.

All 9 patients with primary hypothyroidism responded normally to the

[1] There is a possibility that water intoxication may occur following the administration of 1500 ml. of water. In our experience this complication has rarely been noted. Nonetheless, it is something to be borne in mind during the procedure.

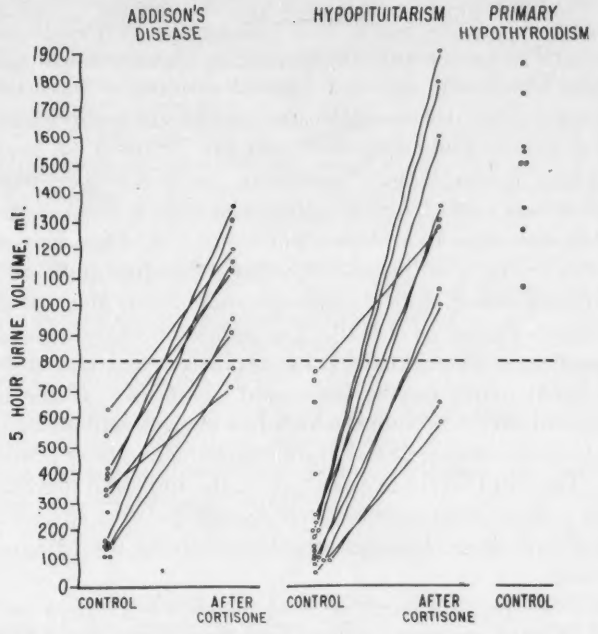

FIG. 1. Results of water loading tests in patients with Addison's disease, hypopituitarism, and primary hypothyroidism.

water loading test. In most of them, specific replacement therapy had been discontinued for only a short period of time, but in others there was a history of untreated hypothyroidism of one to six years' duration (Table 1).

TABLE 1. PRIMARY HYPOTHYROIDISM.
Results of water loading tests

Patient	5-Hour urine volume (ml.)	Duration of disease
1. F.M. ♀	1270	2 yrs. No Rx.
2. M.C. ♀	1560	6 yrs. No Rx. for 3 mos.
3. E.N. ♀	1540	8 yrs. No Rx. for 2 mos.
4. I.G. ♂	1860	3 yrs. No Rx.
5. B.L. ♀	1500	19 yrs. No Rx. for 3 mos.
6. S.C. ♀	1750	2 yrs. No Rx. for 2 wks.
7. I.S. ♀	1070	6 yrs. No Rx.
8. M.S. ♂	1500	1 yr. No Rx. for 7 wks.
9. M.M. ♀	1340	1 yr. No Rx.

DISCUSSION

The abnormal response to water loading in adrenocortical insufficiency is not closely correlated with a decreased glomerular filtration rate, as shown by the fact that desoxycorticosterone and saline can effect a significant increase in the glomerular filtration rate without an improved response to water loading (12). Conversely, cortisone overcomes the abnormal response to water loading, independent of its effect on the glomerular filtration rate (12). In addition, our results and Gaunt's animal work (10, 11) indicate that the diuresis following water loading is not impaired in the hypothyroid state, even though the renal plasma flow and glomerular filtration rate are decreased (13–16). The failure of ethanol to induce water diuresis following a water load in the hypoadrenocortical state (12, 17) would cast great doubt on the functional significance of the increase in circulating antidiuretic hormone which has been described in this condition (18–21), if we assume the antidiuretic hormone to be of posterior pituitary origin. The most likely explanation of the impaired diuretic response in adrenal insufficiency is a specific renal tubular defect in ability to clear filtered water, this defect being overcome rapidly by the administration of glucocorticoids.

Since all of 33 patients with adrenal insufficiency (15 with Addison's disease and 18 with hypopituitarism) who underwent the modified water loading test responded abnormally, followed by substantial improvement in those re-tested during cortisone therapy, the value of the procedure in the diagnosis of the hypoadrenocortical state has been substantiated.

In the 18 patients with hypopituitarism, 13 of whom had unequivocal hypothyroidism, water tolerance was invariably impaired. It would seem likely that the impairment of water tolerance in this disorder is related at least in part to the associated adrenal insufficiency, since the administration of cortisone increased the diuresis.

In patients with primary hypothyroidism water tolerance is unimpaired or, as reported by Crispell *et al.*, if it is impaired, it is not improved after the administration of cortisone. One may conclude that when hypothyroidism is associated with an impaired water tolerance which reverts to normal after the administration of cortisone, the thyroid failure is probably secondary to pituitary failure. Hypothyroidism associated with normal water tolerance is suggestive of primary thyroid dysfunction.

REFERENCES

1. Rosenow, G.: Über die Nierenfunktion bei der Addisonschen Krankheit, *Med. Klin.* **21**: 204, 1925.
2. Rowntree, L., and Snell, A.: A Clinical Study of Addison's Disease. Philadelphia, W. B. Saunders Co., 1931.

3. VITORIA, C.: Metabolismo mineral y del agua en la enfermedad de Addison; la prueba del agua (IV comunicacion), *Arch. de med., cir. y especialid* **38:** 718, 1935.
4. ROBINSON, F.; POWER, M., and KEPLER, E.: Two new procedures to assist in the recognition and exclusion of Addison's disease; a preliminary report, *Proc. Staff Meet., Mayo Clin.* **16:** 577, 1941.
5. OLEESKY, S., and STANBURY, S. W.: Effect of oral cortisone on water diuresis in Addison's disease and hypopituitarism, *Lancet* **2:** 644, 1951.
6. LEWIS, A.; SLESSOR, A.; BURSTON, R., and GARROD, O.: Aspect of renal function in Addison's disease and hypopituitarism, *Proc. Roy. Soc. Med.* **45:** 63, 1952.
7. CHALMERS, T., and LEWIS, A. A. G.: The effect of adrenocorticotropic hormone on the diuretic response to water in panhypopituitarism, *Lancet* **2:** 1158, 1951.
8. SOFFER, L. J., and GABRILOVE, J. L.: A simplified water-loading test for the diagnosis of Addison's disease, *Metabolism* **1:** 504, 1952.
9. CRISPELL, K.; PARSON, W., and SPRINKLE, P.: A cortisone-resistant abnormality in the diuretic response to ingested water in primary myxedema, *J. Clin. Endocrinol. & Metab.* **14:** 640, 1954.
10. GAUNT, R.; CORDSEN, M., and LILING, M.: Water intoxication in relation to thyroid and adrenal function, *Endocrinology* **35:** 105, 1944.
11. GAUNT, R.: Animal experiments relating to water diuresis tests for adrenal insufficiency, *J. Clin. Endocrinol.* **6:** 595, 1946.
12. SOFFER, L. J.; GUTMAN, A.; GELLER, J., and GABRILOVE, J. L.: The role of adrenal steroids on renal function and electrolyte metabolism, *Bull. New York Acad. Med.* **33:** 665, 1957.
13. CORCORAN, A. C., and PAGE, I. H.: Specific renal functions in hyperthyroidism and myxedema, *J. Clin. Endocrinol.* **7:** 801, 1947.
14. HLAD, C. J., JR., and BRICKER, N. S.: Renal function and I^{131} clearance in hyperthyroidism and myxedema, *J. Clin. Endocrinol. & Metab.* **14:** 1539, 1954.
15. COHN, C.: Endocrine influences on renal function, *in* Progress in Clinical Endocrinology, ed. by S. Soskin. New York, Grune and Stratton, 1950, p. 618.
16. YOUNT, E., and LITTLE, J. M.: Renal clearance in patients with myxedema, *J. Clin. Endocrinol. & Metab.* **15:** 343, 1955.
17. LAMDIN, E.; KLEEMAN, C.; RUBINI, M., and EPSTEIN, F.: Studies on alcohol diuresis. III. The response to ethyl alcohol in certain disease states characterized by impaired water tolerance, *J. Clin. Invest.* **35:** 386, 1956.
18. GAUNT, R.; BIRNIE, J., and EVERSOLE, W.: Adrenal cortex and water metabolism, *Physiol. Rev.* **29:** 281, 1949.
19. LLOYD, C. W., and LOBOTSKY, J.: Serum antidiuretic substances and urinary corticosteroid in the human, *J. Clin. Endocrinol.* **10:** 318, 1950.
20. SLESSOR, A.: Studies concerning the mechanism of water retention in Addison's disease and in hypopituitarism, *J. Clin. Endocrinol.* **11:** 700, 1951.
21. BIRNIE, J.; JENKINS, W.; EVERSOLE, W., and GAUNT, R.: An antidiuretic substance in the blood of normal and adrenalectomized rats, *Proc. Soc. Exper. Biol. & Med.* **70:** 83, 1949.

THE EFFECT OF INSULIN ON SERUM LIPOPROTEINS OF DIABETIC PATIENTS*

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ABSTRACT

Serial serum lipoprotein and cholesterol determinations were performed in 17 female diabetic patients institutionalized because of mental illness. Each patient was studied during prolonged periods of low insulin dosage (hyperglycemic but clinically non-acidotic decontrol phase) and high insulin dosage (control phase). Statistical analysis of the data, based on the assay of more than 300 blood samples over 500 patient-weeks of study, showed highly significant increases in $s_f^0$0–12, $s_f^0$100–400, Atherogenic Index and serum cholesterol values in association with the decontrol phase. Mean increases in $s_f^0$0–12 serum lipoprotein and total cholesterol concentrations associated with the decontrol phase were markedly greater in the younger group of patients, although the mean changes in blood sugar concentration were about the same in the 2 age groups (mean ages, 48 and 71 years). The practical significance of the findings is discussed with respect to the biochemical support they lend to the desirability of rigid rather than loose insulin control of diabetic hyperglycemia. Strict long-term control should result in significant lowering of the incidence of serious sequelae of the atherogenic process.

WITH the classification and accurate quantitation of human serum lipoproteins by Gofman and associates (1, 2), renewed interest in serum lipids in the diabetic state led to a reinvestigation of the hyperlipemia of diabetic acidosis. Kolb and Gofman (3) found this hyperlipemia to be characterized by a specific and distinct alteration in the concentrations of both low-density and high-density serum lipoproteins.

Between rigid control of diabetic glycosuria and hyperglycemia with insulin and the complete lack of control manifest in diabetic acidosis and coma, there exists a large area covering cases of diabetes characterized by moderate glycosuria, definite hyperglycemia, but no other signs and symp-

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toms. Many of the insulin-requiring adult diabetics treated by physicians favoring "loose" control of the diabetic state, would probably fall in this category. The present report concerns a controlled study of the serum lipoprotein concentrations associated with rigid versus loose control of the diabetic state with insulin.

SUBJECTS AND PROCEDURE

The subjects of the present study were a group of 17 female chronic psychiatric patients hospitalized on the medical service of a state mental institution because of diabetes mellitus. Pertinent clinical data on these patients are recorded in Table 1.

All patients were ambulatory diabetics who were not known to have any other acute or chronic disease of significant degree. Patients in whom the initial diagnosis had been hypertensive cardiovascular disease (HCVD) with or without congestive heart failure (CHF) were well compensated during the period of this study. All patients had diabetes of the stable type, as defined by Alivisatos and McCullagh (4) based on blood-sugar distribution patterns and absence of ketotic and insulin reactions. No exact information of the duration of diabetes was available in each case but, in general, these patients had been known diabetics for many years. All of them were given the same type of hospital diet for diabetic patients, consisting of 2200 calories, with approximately 90 grams of protein, 100 grams of fat and 220 grams of carbohydrate. The total daily intake was divided as follows: 1/7 (breakfast), 1/7 (mid-morning snack), 2/7 (lunch), 1/7 (mid-afternoon snack), 2/7 (dinner).

Individual patients were studied for periods varying from twelve to forty-seven weeks and serial blood samples were obtained in each patient during periods of relatively low and high insulin dosage. Thus, for each patient blood samples obtained during hyperglycemic, but not acidotic, periods (subsequently referred to as *'decontrol"* samples) could be compared with serial samples collected during prolonged relatively normoglycemic intervals (subsequently referred to as *"control"* samples).

Complete analyses of serum for both low-density and high-density lipoproteins were performed on each sample (5), as were determinations of total serum cholesterol (6) and fasting blood sugar (7).

All blood samples were drawn in the morning before breakfast and before injection of the daily dose of PZI insulin. Body weight, blood pressure and pulse rate were recorded at the time of venipuncture.

RESULTS

In Table 2 a concise summary is presented of the data obtained in individual patients. Mean control and decontrol levels of lipoproteins of all classes and of total serum cholesterol are recorded, as are mean fasting blood sugar levels, insulin dosage and body weight. Also shown are the duration (in weeks) of both control and decontrol phases for each patient and the number of blood samples obtained during these periods.

The statistical significance of the observed differences in mean serum lipoprotein levels between control and decontrol phases was evaluated in each patient by calculation of t-values based on t-tests for differences in uncorrelated means. Use of this statistic rather than that for differences in

TABLE 1. SUMMARY OF CLINICAL DATA (17 PATIENTS)

Patient & Age (yrs.)	Psychiatric diagnosis	Medical diagnoses (other than diabetes mellitus)	Medication and dosage in grams
A.L. 46	CBS* with psychotic reaction	Chronic alcoholism with polyneuritis, cirrhosis and multiple vitamin defic.	Banana b.i.d.
C.F. 66	CBS with psychosis	HCVD†, compensated	None
C.A. 72	Schizophrenic reaction, hebe.	Blindness, bilat.	Dig.§, 0.100 q.d.
C.M. 83	Schizophrenic reaction, hebe.	HCVD with mild CHF‡	Dig., 0.100 q.d.
C.R. 60	Manic depressive reaction, manic	HCVD with CHF, compensated; sinus tachycardia	Dig., 0.100 q.d.
C.A. 43	Schizophrenic reaction, catatonic		Thorazine, 0.300 q.i.d.
E.M. 64	Schizophrenic reaction, hebe.		Dig., 0.100 q.d.; Thorazine
A.A. 58	Involutional psychosis, paranoid		Dig., 0.100 q.d.
H.M. 75	Senile psychosis; CBS with senile brain disease	HCVD; old rt. hemiplegia	Dig., 0.100 q.d.
J.M. 45	Schizophrenic reaction, paranoid	HCVD, compensated; uterine fibroids; treated syphilis	Dig. 0.100 q.d.; Thorazine, 0.200–0.300 b.i.d.
J.G. 65	Schizophrenic reaction, hebe.	Obesity; HCVD with CHF; convulsive seizures controlled with Dilantin	Dig., 0.100 q.d.; Dilantin, 0.100 b.i.d.
L.J. 65	Manic depressive reaction, depressive type	Obesity	None

* CBS = chronic brain syndrome.

† HCVD = hypertensive cardiovascular disease.

‡ CHF = congestive heart failure.

§ Dig. = digitalis leaf.

TABLE 1.—*Continued*

Patient & Age (yrs.)	Psychiatric diagnosis	Medical diagnoses (other than diabetes mellitus)	Medication and dosage in grams
M.M. 87	CBS with senile brain disease and with psychotic reaction	HCVD with early CHF; dilat. cataracts; obesity	Dig., 0.100 q.d.
O.M. 45	Schizophrenic reaction; manic depressive reaction, manic type	Pulm. tuberculosis, inactive; HCVD, compensated	None
S.M. 54	Mental deficiency, idiopathic	Enlarged thyroid gland; squamous-cell carcinoma in situ (removed by conization)	Thorazine, 0.100 b.i.d. Artane, 0.005 q.d.
T.E. 57	Involutional psychotic reaction	Mild HCVD; moderate obesity	None
Y.S. 44	Schizophrenic reaction, chronic undiff'd type		Ritalin, 0.010 q.d. Thorazine, 0.100 b.i.d.

correlated means has the effect of underestimating the actual significance of these differences (8). The *P* values recorded in Table 2 therefore represent a conservative estimate of the significance of the differences between means.

Inspection of Table 2 shows the following: 1) In almost all patients, serum lipoprotein levels and Atherogenic Index (A.I.)[1] values (9) tend to be higher in the decontrol phase than in the control phase, although there are fairly large variations in the magnitude of these changes in individual patients; 2) of the various serum lipid measurements performed quantitatively, the largest response to insulin is observed in the $s_f^\circ 0–12$ class, followed by that of the serum cholesterol; 3) no consistent changes are evident in $s_f^\circ 12–20$, in $s_f^\circ 20–100$ and in HDL-3, the major high-density serum lipoproteins.

Analysis of these data for the group of patients as a whole (Table 3) confirms and quantitates the foregoing statements. This table shows the remarkable consistency and highly significant increase in $s_f^\circ 0–12$ concentration associated with decontrol of the diabetic state. Total serum cholesterol levels showed the next largest increase, followed by $s_f^\circ 100–400$ levels and Atherogenic Index values. No significant changes in $s_f^\circ 12–20$ and in HDL-3 concentrations occurred and only small increases of questionable statistical significance were observed in the $s_f^\circ 20–100$ lipoproteins.

[1] A.I. $= 0.1(\text{mg. } s_f^\circ 0–12) + 0.175(\text{mg. } s_f^\circ 12–400)$.

TABLE 2. SUMMARY OF DATA FOR INDIVIDUAL PATIENTS

Patient & Age (yrs.)	Insulin dosage (units)	N*	D* (wks.)	Fasting blood sugar (mg./100 ml.)		Serum lipoproteins (mg./100 ml.)						Serum cholesterol (mg./100 ml.)	Body wt. (lbs.)
				Mean	Range	S_f 0–12	S_f 12–20	S_f 20–100	S_f 100–400	A.I. (units)	HDL–3 (mg./100 ml.)		
A.L. 46	(45–50)C†	4	3	82	57–100	247	55	58	9	46		176	95
	(20–35)DC†	12	18	252	93–385	292	54	72	11	53		199	93
						P<0.10	NS‡	NS	NS	NS		P~0.10	
C.F. 66	(40–45)C	11	8	105	56–212	583	128	115	27	106	270	369	126
	(15–40)DC	13	29	217	63–330	613	125	141	24	112	226	360	119
						NS	NS	NS	NS	NS	P<0.001	NS	
C.A. 72	(55–65)C	12	21	103	41–190	317	42	62	17	53	200	196	133
	(10–30)DC	9	13	217	131–285	299	45	83	19	56	196	197	128
						NS	NS	NS	NS	NS	NS	NS	
C.M. 83	(30–65)C	9	15	130	71–157	323	81	90	10	64	205	213	141
	(none)DC	4	5	179	137–279	325	70	68	7	58	223	209	139
						NS	NS	NS	NS	NS	NS	NS	
C.R. 60	(40–95)C	9	16	115	80–156	425	80	81	18	74	267	276	118
	(none)DC	8	11	195	170–226	447	82	70	25	76	301	301	116
						NS	NS	NS	NS	NS	NS	P~0.05	
C.A. 43	(25–40)C	9	18	74	36– 91	379	120	87	14	77	308	307	117
	(none)DC	9	15	95	64–126	457	114	121	17	90	315	338	117
						P<0.01	NS	P=0.05	NS	P<0.05	NS	P<0.10	
E.M. 64	(35–55)C	10	22	85	57–107	299	67	195	36	79	160	198	99
	(0–20)DC	13	25	165	114–201	325	59	209	68	91	174	220	104
						P<0.10	NS	NS	P<0.02	P<0.05	NS	P~0.02	
A.A. 58	(30–45)C	6	7	117	76–172	419	47	39	4	65	206	258	119
	(0–25)DC	8	15	206	151–267	473	58	55	12	89	251	285	120
						P<0.10	NS	NS	NS	P<0.10	P~0.01	P~0.05	
H.M. 75	(40–45)C	15	24	118	68–187	493	82	95	29	85	191	289	128
	(none)DC	16	16	290	206–333	496	80	107	44	90	207	293	120
						NS	NS	NS	NS	NS	NS	NS	

TABLE 2. *Continued*

Patient & Age (yrs.)	Insulin dosage (units)	N*	D* (wks.)	Fasting blood sugar (mg./100 ml.)		Serum lipoproteins (mg./100 ml.)						Serum cholesterol (mg./100 ml.)	Body wt. (lbs.)
				Mean	Range	S_f 0–12	S_f 12–20	S_f 20–100	S_f 100–400	A. I. (units)	HDL-3 (mg./100 ml.)		
J.M. 45	(75–130)C	8	13	90	39–194	444	82	99	23	80	216	270	181
	(30–45)DC	8	15	244	130–289	496	88	131	50	97	256	305	179
						P~0.05	NS	NS	P <0.05	P <0.10	P <0.02	P <0.05	
J.A. 65	(10–20)C	10	19	80	70– 95	407	78	97	23	75	233	280	144
	(none)DC	10	16	100	79–126	415	72	117	52	84	205	275	143
						NS	NS	NS	P~0.05	NS	P <0.05	NS	
L.J. 65	(20–35)C	7	2	117	74–143	391	78	94	24	73	251	238	139
	(none)DC	20	18	148	106–183	397	71	89	34	74	246	253	138
						NS	NS	NS	NS	NS	NS	NS	
M.M. 87	(30–60)C	15	30	112	60–139	281	68	94	27	62	227	218	131
	(none)DC	8	8	178	114–231	315	60	79	28	61	246	230	135
						P <0.02	NS	NS	NS	NS	NS	NS	
O.M. 45	(30)C	10	17	86	67–151	367	70	83	27	68	312	261	112
	(none)DC	9	19	145	120–174	403	68	111	52	81	309	288	114
						P <0.05	NS	P <0.05	P~0.10	P <0.05	NS	P <0.01	
S.M. 43	(65–110)C	6	9	123	75–175	429	71	126	52	86		263	205
	(40–55)DC	11	18	211	162–232	490	73	137	70	98		293	207
						P <0.02	NS	NS	P <0.05	P~0.02		P~0.02	
T.E. 57	(30)C	4	3	95	85–104	341	56	54	7	55	201	213	132
	(none)DC	8	9	148	89–187	359	49	48	7	54	205	232	129
						NS	NS	NS	NS	NS	NS	P <0.05	
Y.S. 44	(65–105)C	5	6	150	115–195	407	43	110	44	75		256	147
	(30–45)DC	12	18	250	226–270	448	46	87	30	73		257	146
						P~0.10	NS	P<0.05	NS	NS		NS	

* N refers to the total number of blood samples obtained in each patient in a given phase.
D indicates the duration of the phase in weeks.
† C and DC identify the control and decontrol phases, respectively.
‡ NS = not significant.

TABLE 3. SIGNIFICANCE OF CHANGES IN SERUM LIPOPROTEINS, ATHEROGENIC INDEX, AND TOTAL CHOLESTEROL OBSERVED IN THE ENTIRE GROUP OF 17 PATIENTS

Measure	Mean control level*	Mean decontrol level†	Difference	Significance of the difference	
				t-value	P-value
s_f°0–12 (mg./100 ml.)	385	415	+30	4.90	<0.001
s_f°12–20 (mg./100 ml.)	73	71	− 2		not signif.
s_f°20–100 (mg./100 ml.)	93	101	+ 8	1.90	<0.10
s_f°100–400 (mg./100 ml.)	23	32	+ 9	2.78	<0.02
Atherogenic Index (units)	72	79	+ 7	3.84	<0.01
HDL-3 (mg./100 ml.)	232	240	+ 8		not signif.
Cholesterol (mg./100 ml.)	252	267	+15	4.34	<0.001
FBS (mg./100 ml.)	105	191	+86	11.30	≪0.001
Insulin dosage (units)	49	13	−36	7.23	≪0.001

* Mean value of 150 blood samples representing a study-period of 229 patient-weeks.
† Mean value of 178 blood samples representing a study-period of 268 patient-weeks.

To evaluate the effect of age on the serum lipoprotein changes associated with diabetic control versus decontrol, the 17 patients were divided into 2 subgroups comprising the 9 oldest patients (mean age 70.6 years) and the 8 youngest patients (mean age 48.0 years), following which serum lipoprotein data were computed for each subgroup. The results of this analysis (Table 4) show clearly that diabetic control produces a distinctly greater reduction of s_f°0–12 serum lipoprotein and cholesterol levels and of A.I. values in the younger age group. The fact that the Δ-insulin and Δ-FBS values are comparable in the 2 age groups rules out the possibility that the greater changes in s_f°0–12 and cholesterol levels observed in the younger

TABLE 4. EFFECT OF AGE ON THE SERUM LIPOPROTEIN RESPONSE TO INSULIN

	Old group	Young group	Significance of differences in Δ-values
No. of patients	9	8	
Mean age (yrs.)	70.6	48.0	
Age range (yrs.)	60–87	43–58	
Δ-s_f°0–12 (mg./100 ml.)	13	48	P∼0.01
Δ-s_f°100–400 (mg./100 ml.)	10	9	not signif.
Δ-Atherogenic index (units)	3	9	P<0.10
Δ-Cholesterol (mg./100 ml.)	9	24	P<0.02
Δ-FBS (mg./100 ml.)	80	92	not signif.
Δ-Insulin dosage (units)	43	38	not signif.

TABLE 5. LIST OF PAIRS OF VARIABLES WITH NON-SIGNIFICANT CORRELATION COEFFICIENTS

For entire group	Initial $s_f^0$0–12	*vs.*	Δ-$s_f^0$0–12*
	Initial A.I.	*vs.*	Δ-A.I.
	Initial FBS	*vs.*	Decontrol A.I.
	Δ-FBS	*vs.*	Δ-A.I.
	Δ-Insulin	*vs.*	Δ-A.I.
For younger age group only	Δ-FBS	*vs.*	Δ-A.I., Δ-$s_f^0$0–12, Δ-$s_f^0$100–400
	Δ-Insulin	*vs.*	Δ-A.I., Δ-$s_f^0$0–12, Δ-$s_f^0$100–400

* Δ refers to: (mean value in decontrol phase) − (mean value in control phase).

patients are due to a greater increment in insulin dosage in this group or to a greater mean change in FBS between control and decontrol levels.

To ascertain the possible existence of factors other than age which might influence the serum lipoprotein response to insulin, various correlation coefficients were calculated. However, no statistically significant correlations were discovered between such variables as initial $s_f^0$0–12 levels and Δ-$s_f^0$0–12, initial A.I. and Δ-A.I., initial FBS and decontrol-A.I., ΔFBS and Δ-A.I., Δ-insulin and Δ-A.I., and others summarized in Table 5.

DISCUSSION

It is of interest to compare the present findings with those reported for diabetic acidosis by Kolb, deLalla and Gofman (3). For this purpose, 5 female patients from their series who were in a state of severe diabetic acidosis were matched in age and sex with 5 female patients of the present series (in the decontrol state) and with 5 normal controls. The data so obtained are summarized in Table 6.

A perhaps unexpected aspect of these data is the elevation of the $s_f^0$0–12 level in non-acidotic but decontrolled diabetics compared to the marked lowering of this level in the severely acidotic state, the latter being presumably associated with an even greater lack of insulin than the decontrolled but non-acidotic state. This observation is in line, however, with the observation of Kolb *et al.* (3) of a marked increase in $s_f^0$0–12 serum lipoprotein concentration—far above normal levels—during the early phase of recovery from a very severe acidotic state. Thus the serum lipoprotein distribution pattern found in mild acidosis and that associated with decontrolled but non-acidotic diabetic hyperglycemia show rising $s_f^0$20–100 and $s_f^0$100–400 levels with increasing acidosis; only in very severe, untreated acidosis is the additional feature of a reversal of $s_f^0$0–12 concentrations to markedly subnormal levels observed. A pH effect on enzyme-

TABLE 6. COMPARISON OF SERUM LIPOPROTEIN CONCENTRATIONS IN DECONTROLLED NON-ACIDOTIC AND IN SEVERELY ACIDOTIC DIABETES

	5 Female Diabetics (decontrol phase non-acidotic)	5 Female Diabetics (with acidosis; 2 in coma)	5 Normal Females
Mean age (yrs.)	45.8	42.2	45
s_f°0–12 (mg./100 ml.)	453	270	370
s_f°12–20 (mg./100 ml.)	63	89	60
s_f°20–100 (mg./100 ml.)	92	502	66
s_f°100–400 (mg./100 ml.)	34	690	22
HDL-1 (mg./100 ml.)*	21	111	23
HDL-2 (mg./100 ml.)*	115	38	89
HDL-3 (mg./100 ml.)*	270	93	241

* Mean values for 4 patients.

mediated reactions controlling s_f°0–12 serum lipoprotein concentrations may account for this phenomenon.

The practical significance of the findings in this study relate to the biochemical basis they provide for the assertion that the diabetic state should be well controlled rather than loosely controlled. Because of the proven statistical association of elevated A.I. values with increased risk from coronary artery disease (10, 11), long-term careful control of the diabetic state should reduce the mortality from coronary artery disease. This applies particularly to younger diabetic patients. In these, a greater reduction in serum lipoprotein levels and A.I. values may be expected. As coronary atherogenesis is a cumulative time-integrated process, a greater chance of slowing, stopping or perhaps of even reversing this process exists.

Extension of the present study to include male and female diabetics of all age groups and to diabetic populations maintained with oral hypoglycemic agents appears well justified by the importance of this problem. The absence of such information to date is undoubtedly due to the great practical difficulty of studying diabetic patients for prolonged periods during deliberately poorly controlled hyperglycemic phases while simultaneously maintaining very close medical supervision. The medical ward of a large state mental institution provided the unusual conditions which made the present study possible.

Acknowledgment

We gratefully acknowledge the cooperation of Dr. W. A. Oliver, Director of Clinical Services, and of the ward personnel of the Napa State Hospital, Imola, California, of the Department of Mental Hygiene of the State of California. We also wish to thank

the technical workers of the Donner Laboratory for the ultracentrifugal and cholesterol analyses.

REFERENCES

1. GOFMAN, J.; LINDGREN, F., and ELLIOTT, H.: Ultracentrifugal studies of lipoproteins of human serum, *J. Biol. Chem.* **179**: 973, 1949.
2. GLAZIER, F.; TAMPLIN, A.; STRISOWER, B.; DELALLA, O.; GOFMAN, J.; DAWBER, T., and PHILLIPS, E.: Human serum lipoprotein concentrations, *J. Gerontol.* **9**: 395, 1954.
3. KOLB, F. O.; DELALLA, O. F., and GOFMAN, J. W.: The hyperlipemias in disorders of carbohydrate metabolism: serial lipoprotein studies in diabetic acidosis with xanthomatosis and in glycogen storage disease, *Metabolism* **4**: 310, 1955.
4. ALIVISATOS, J. G., and MCCULLAGH, E. P.: Stable and brittle diabetes, *Am. J. Med.* **21**: 344, 1956.
5. DELALLA, O., and GOFMAN, J.: Ultracentrifugal analysis of human serum lipoproteins, *in* Methods of Biochemical Analysis, ed. b Glick, D. New York, Interscience Publishers, Inc., Vol. 1, 1954.
6. COLMAN, D. M., and MCPHEE, A.: An improved method for the determination of total serum cholesterol, *Am. J. Clin. Path.* **26**: 181, 1956.
7. FOLIN, O.: Two revised copper methods for blood sugar determinations, *J. Biol. Chem.* **82**: 83, 1929.
8. GUILFORD, J. P.: Fundamental Statistics in Psychology and Education, ed. 3. New York, McGraw-Hill Book Co., Inc., 1956, pp. 219–220.
9. GOFMAN, J. W.; STRISOWER, B.; DELALLA, O.; TAMPLIN, A.; JONES, H. B., and LINDGREN, F.: Index of coronary artery atherogenesis, *Modern Med.* pp. 119–140, June 15, 1953.
10. GOFMAN, J. W.; HANIG, H.; JONES, H. B.; LAUFFER, M.; LOWRY, E.; LEWIS, L.; MANN, G.; MOORE, F.; OLMSTEAD, F., and YEAGER, F.: Evaluation of serum lipoprotein and cholesterol measurements as predictors of clinical complications of atherosclerosis. Report of a cooperative study of lipoproteins and atherosclerosis, *Circulation* **14**: 691, 1956.
11. GOFMAN, J. W.: Serum lipoproteins and the evaluation of atherosclerosis, *Bull. New York Acad. Sc.* **64**: 590, 1956.

Letters to the Editor

COLLABORATIVE ASSAYS OF TWO LOCAL STANDARDS FOR HUMAN PITUITARY GONADOTROPIN

To the Editor:

In a previous collaborative study (1), data were presented to show that the establishment of an international standard preparation of human pituitary gonadotropin (HPG) would be feasible and highly desirable. These data consisted of collaborative assays of HPG prepared from urine of menopausal-postmenopausal women and from male urine, both preparations being made by similar, although not identical, kaolin-acetone methods.

The present study contains collaborative assays of the same local standard of male urine (AMW) that was used in the previous study and of another local standard made by ultrafiltration from ovariectomized women's urine (OWU). This study, therefore, enlarges the scope of the collaborative work in that another source (castrated women) for HPG was employed and another method (ultrafiltration) for preparing HPG was used.

The preparation of the local HPG standard from the urine of men (AMW) has been described previously (2). It was prepared by a kaolin-acetone procedure and further purified by alcohol fractionation; 1 mg. of this preparation contains the gonadotropic activity of approximately 50 ml. of the original urine. The local HPG standard (OWU) from the urine of castrated women was prepared in Detroit (Wayne State University) by the ultrafiltration process (3). Collections of urine (twenty-four hours) from 5 women castrated from eight months to six years previously and ranging in age from 27 to 50 years were processed and pooled; 1 mg. of the pooled preparation contained the gonadotropic activity of approximately 11 ml. of original urine.

Two assay systems were used as follows:

Ovarian-weight method in intact rats. In Rochester, the ovarian-weight method in intact rats was employed in a three-and-three assay design (3 doses of one standard and 3 doses of the other standard) using 5 rats per dose. Once a day for four days, each animal received an injection of 2 ml. of material. Necropsy was performed on the fifth day. The rats (Sprague-Dawley strain) were 21 to 23 days old at the time of the first in-

jection and weighed from 38 to 50 Gm. In Detroit (Wayne State University), the ovarian-weight method in intact rats also was employed, usually in a four-and-four design using 3 rats per dose. These animals were also of the Sprague-Dawley strain but were 25 days of age at the start of the assay. The animals received injections of 1 ml. twice daily for three days and were examined twenty-four hours after the last injection.

Uterine-weight method in intact mice. In Detroit (Henry Ford Hospital), the assay system used was the uterine-weight method in intact mice. A three-five and three-five assay design employing 10 mice per dose was used.

TABLE 1. COLLABORATIVE ASSAY OF OWU IN TERMS OF AMW*

End point	Place	Design	N	λ	R.P.	Confidence limits†
Ovarian weight, intact rats	Rochester	2 & 2	20	0.21	3.2	(1.9–8.6)
		3 & 3	30	0.11	3.6	(3.0–4.4)
Ovarian weight, intact rats	Detroit (Wayne State)	3 & 3	18	0.34	5.7	(2.4–27.0)
		3 & 2	15	0.08	7.8	(6.3–12.2)
Uterine weight, intact mice	Detroit (Henry Ford)	2 & 3	47	0.10	2.7	(2.2–3.2)
		2 & 2	30	0.09	3.1	(2.6–4.1)
		2 & 2	36	0.06	3.1	(2.8–3.5)

* N = total number of animals; λ = index of precision; R.P. = relative potency, or units per milligram, 1 unit being the activity contained in 1.0 mg. of AMW.

† P = 0.95.

Six injections of 0.1 ml. were given to each animal. The schedule started in the afternoon of the first day with 1 injection. Two injections were given on each of the second and third days, and the sixth injection was given on the morning of the fourth day. Necropsy was performed on the fifth day of the assay.

The results obtained are shown in Table 1. The calculations employed are those described by Borth and associates (4). Examination of the individual values of relative potency indicates good agreement between the values obtained in Rochester and those at the Henry Ford Hospital, utilizing 2 different methods of biologic assay. This is confirmatory of the aforementioned first collaborative study, which showed close agreement between the ovarian-weight method in rats and the uterine-weight method in mice. However, the comparison of relative potency obtained at Rochester and at Wayne State University, utilizing the ovarian-weight method of assay in intact rats at both places, showed some divergence. Accordingly, several estimates of the relative potency were obtained.

TABLE 2. ESTIMATES OF RELATIVE POTENCY OF OWU IN TERMS OF AMW

Place	Original design	N*	Relative potency†			Mean
			Graphic	Algebraic	Orthogonal	
Rochester	3 & 3	30	3.7	3.4	3.2	3.54
	3 & 3	30	3.7	3.6	3.6	
Detroit (Wayne State)	4 & 4	24	4.4	4.8	5.7	5.85
	4 & 2	18	6.0	6.4	7.8	
Detroit (Henry Ford)	4 & 3	67	3.3	3.6	2.7	3.14
	3 & 5	49	3.2	3.1	3.1	
	3 & 3	55	3.1	3.1	3.1	

* Total number of animals.

† Units per milligram, 1 unit being the activity contained in 1.0 mg. of AMW.

Table 2 shows 3 estimates of relative potency of each assay, each estimate made independently by a different method. In this table, the original assay design is indicated, since all values obtained could be used for estimation of relative potency by the graphic and algebraic methods but not by the orthogonal method. In the graphic method, parallel lines that best fitted the points visually were laid down among the points for each of the 2 preparations. The distance on the log scale between these 2 lines is a simple estimation of relative potency. The algebraic and orthogonal methods for calculating the assay require no further description. It is to be noted that the relative potency estimated by all 3 methods agreed well for the data obtained in Rochester and for those obtained at the Henry Ford Hospital. The relative potency obtained at Wayne State University by the orthogonal method was greater than that obtained by the graphic and algebraic methods. Therefore, the mean relative potency for each assay system was determined. These means were used to determine the mean index of discrimination (Table 3).

The data in Tables 1, 2 and 3 were analyzed by methods described in

TABLE 3. MEAN INDEX OF DISCRIMINATION FOR 3 ASSAYS OF HPG

Combination of assays	Mean index of discrimination
Rat ovary (Detroit)/rat ovary (Rochester)	1.65
Rat ovary (Detroit)/mouse uterus (Detroit)	1.85
Rat ovary (Rochester)/mouse uterus (Detroit)	1.12

the previous study. The mean unweighted confidence limits (approximately 3 to 9) embrace, or are close to, all values of relative potency obtained. Thus, the span between the lowest and highest value of relative potency (2.7 to 7.8) is less than the span between the mean upper and lower confidence limits. Therefore, the individual assays may be regarded as fluctuating within the range predicted by the internal estimate of error. The mean index of discrimination did not differ greatly from a value of 1. The average of the mean index of discrimination for the 2 combinations of rat ovary/mouse uterus was 1.48. The similar value for the rat ovary /mouse uterus combination in the previous report was 1.45. Thus, good agreement in this respect is present between the 2 studies.

The differences in results are chiefly those between the 2 laboratories using the ovarian-weight method in intact rats. These differences apparently result from variations in assay technic and design. The agreement between the Rochester assays in rats and the Detroit assays in mice is impressive. This would indicate that differences in the chemical and physical state of the 2 preparations, as must certainly exist between AMW and OWU, play little role in causing variation in results. They also indicate that, regardless of differences in the qualitative nature of the inert material present in these 2 preparations, the sum total of solids given to the animals plays little role in producing fluctuation, since the amount of AMW injected was four times as great as that of OWU.

The present data confirm the feasibility of the use of an international standard preparation of HPG. The reservations described in the previous study are reiterated, since the present data do not have bearing on the question of whether qualitative differences are present in HPG of men and of women. Moreover, differences in ratios of follicle-stimulating, luteinizing and luteotropic hormones, if indeed such are present in the 2 standards, do not appear to be detectable by the assay systems used. Regardless of this question, the data do show that at present it is likely that HPG can be measured in terms of an international standard preparation by either the mouse uterine-weight method or the rat ovarian-weight method, the 2 assay methods most widely used for routine clinical purposes.

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REFERENCES

1. ALBERT, A.; BORTH, R.; DICZFALUSY, E.; LORAINE, J. A.; LUNENFELD, B.; MCARTHUR, J. W., and ROSEMBERG, E.: Collaborative assays of two urinary preparations of human pituitary gonadotropin (Letter to the Editor), *J. Clin. Endocrinol. & Metab.* **18:** 1117–1123 (Oct.) 1958.
2. ALBERT, A.: Preparation of a provisional standard for urinary gonadotropin of men, *Proc. Staff Meet., Mayo Clin.* **31:** 341–344 (May 30) 1956.
3. GORBMAN, A.: Ultrafiltration of urine for collection and biological assay of excreted hypophyseal hormones, *Endocrinology* **37:** 177–190 (Sept.) 1945.
4. BORTH, R.; DICZFALUSY, E., and HEINRICHS, H. D.: Grundlagen der statistischen Auswertung biologischer Bestimmungen, *Arch. Gynäk.* **188:** 497–538 (July 10) 1957.

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DETERMINATION OF SEX CHROMATIN IN THE POLYMORPHONUCLEAR NEUTROPHILS OF ENRICHED SMEARS

TO THE EDITOR:

Interest continues to grow in the determination of genetic sex through the study of sex chromatin.

Although identification of sex chromatin in the blood smear is easy, and the method of staining is universally used (1, 2), it takes one or more hours to count 500 polymorphonuclear neutrophils. Thus the method is excessively slow, consuming hours of the investigator's time for a single examination. This fact limits the usefulness of the method.

It was decided to modify the original technique, so as to concentrate the leukocytes. As a result of this change we are now able to count 500 polymorphonuclear neutrophils and identify the sex chromatin in from ten to twenty minutes.

METHOD

1) A sample of blood (about 2 ml.) is obtained by venous puncture, and placed in a tube containing Paul-Heller anticoagulant solution.

2) A 1-ml. portion of this blood is transferred to a Wintrobe hematocrit tube with a capillary pipette, and centrifuged at 1000 revolutions for five minutes.

3) The supernatant plasma is carefully drawn off, taking care not to aspirate the upper part of the centrifuged material. The plasma may be aspirated with the same pipette, as Wintrobe advises (3), or adapted to a glass syringe lubricated with vaseline or silicone.

4) By gentle aspiration, a small sample of the centrifuged blood cells is extracted.

5) This sample is spread on a new, clean plate and stained by the Wright or May-Gruenwald Giemsa method.

6) Counting is performed at a magnification of 600 to 1000.

With the described technique, there is an appreciable enrichment of the proportion of leukocytes in the smear and the polymorphonuclear neutrophils retain their morphology. Because of their greater number, it is possible to select those of more perfect form and to consider only those with nuclei containing more than 3 lobules.

TABLE 1. PERCENTAGE OF 500 POLYMORPHONUCLEAR LEUKOCYTES WITH NUCLEI CONTAINING FEMALE SEX CHROMATIN

	Case number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ordinary blood smear	1.2	2.0	0	0	0	0	2.4	3.8	1.2	1.6	2.4	1.8	1.4	2.0	0	0.8
Enriched blood smear	1.2	2.8	0	0	0	0	1.8	4.0	2.2	1.4	3.4	1.6	1.6	2.2	0	1.2

In 16 normal subjects, a comparative study was made, using an ordinary smear and an enriched smear from the same blood. The findings demonstrated that the new method does not alter the form or reduce the quantity of nuclear female chromatin (Table 1).

Female chromatin, when present, is found in the nuclei of mature polymorphonuclear neutrophils. The method described permits selection of a larger number of leukocytes with well-segmented nuclei. This explains such differences in results as those observed in Cases 2, 9 and 11. A larger number of mature cells counted means a greater probability of identifying more sex chromatin.

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REFERENCES

1. Davidson, W. M., and Smith, D. R.: A morphological sex difference in the polymorphonuclear neutrophil leucocytes, *Brit. M. J.* **2**: 6, 1954.
2. Briggs, D. K., and Kupperman, H. S.: Sex differentiation by leukocyte morphology, *J. Clin. Endocrinol. & Metab.* **16**: 1163, 1956.
3. Wintrobe, M. M.: Clinical Hematology, ed. 3. Philadelphia, Lea & Febiger, 1953, p. 303.

SPONTANEOUS FORMATION OF FERN-LEAF PATTERNS IN HUMAN SALIVA

To the Editor:

Cyclic changes in the fern-leaf crystallization of cervical mucus have been observed and used for diagnostic purposes by many authors. Recently, Andreoli and Della Porta (1) described in this Journal the same tendency to form fern-like patterns in human saliva and found a correlation with the estrogenic phase of the menstrual cycle. It was said that after ovulation, in the second half of the regular cycle, this formation does not occur.

We have examined the saliva, dried at room temperature: *a*) of 10 normal men, *b*) of 8 women with regular menses, from the fourth to the second day before the onset of menstrual bleeding, and *c*) of 2 pregnant women. In all these cases, similar typical fern-leaf patterns were found.

Further, we studied the eventual effect of the pH of the saliva on this crystallization, by rinsing the mouth with both acid and alkaline solutions. No significant difference in crystal formation was found, though at low pH values the pattern was somewhat thicker.

It seems that the tendency to formation of fern-leaf patterns in dried saliva bears no relationship to sex, the phase of the menstrual cycle or estrogen levels, but is a property of the salivary mucus.

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REFERENCE

1. Andreoli, C., and Della Porta, M.: Cyclic changes in female saliva, *J. Clin. Endocrinol. & Metab.* **17**: 913 (July) 1957.

ORAL RELEASIN IN DYSMENORRHEA

To the Editor:

It has been reported that Releasin®, a purified preparation of the ovarian hormone, relaxin, when given intramuscularly or intravenously can halt premature labor apparently by interfering with uterine contractions (1). Further, it has been suggested by Dr. R. D. Sharper, Medical Director at Warner-Chilcott Laboratories, that contrary to expectations Releasin is active by mouth and might prove useful for the control of intractable dysmenorrhea.[1] Accordingly, we tested this preparation in 24 young women in whom the diagnosis of primary dysmenorrhea had been made.

The diagnosis of primary dysmenorrhea was considered established after any evidence of organic disease had been excluded by means of complete history and physical examination with appropriate laboratory work. Only those patients with severe dysmenorrhea (such that classes were regularly missed for one day or longer) were included in this study.

This investigation was carried out using the "double blind" technique. According to a schedule of randomization of cases, the drugs or placebos were dispensed by one of us (A.C.), who had no direct contact with the patients. The schedule was unknown to the other investigators. Medication was available in identical tablets which contained either a placebo, 2 mg. of Releasin, or 5 mg. of Releasin.[2] The drug or placebo was always administered in the same way—one tablet orally every hour after the onset of menstrual pain for four doses, except that when a dosage of 15 mg. was used, three 5-mg. tablets were given every hour for four doses. For easy identification these regimens have been labeled placebo (P), drug 2-mg. (D2), and drug 15-mg. (D15).

Sixteen case studies were performed in patients who, on an alternate monthly basis, were randomized with regard to regimens D2 and P. Eight case studies were performed on patients who were randomized with regard to regimens D2, P and D15. The results are summarized in Tables 1 and 2.

Although the series of patients is small, the results indicate that even with dosages as high as 15 mg. every hour for four doses the patients either obtained no relief or were unable to distinguish between the placebo and the medication. The latter statement receives support from the fact that, in the first group of 16 patients, only 2 felt that the drug had greater effect than the placebo, and in the second group of 8 patients, only 1 noted

[1] Since submission of this letter for publication, Abramson and Reid's report on this point (2) has come to our attention. Their findings are the opposite of ours.

[2] The drug and placebo were furnished by Dr. Richard X. Sands of the Warner-Chilcott Laboratories, Morris Plains, N. J.

TABLE 1. EFFECT OF REGIMENS D2 AND P*

	No. of cases
No relief with either D2 or P	7
Relief with both D2 and P	4
Relief with D2 but none with P	2
Relief with P but none with D2	3
Total	16

* D2 refers to drug administered in dosage of 2 mg. every hour for four hours.
P refers to placebo administered in an identical manner.

TABLE 2. EFFECT OF REGIMENS D2, P AND D15*

	No. of cases
No relief with D2, P, or D15	3
Relief with D2 but not with P or D15	1
Relief with P but not with D2 or D15	1
Relief with D15 but not with D2 or P	0
Relief with D2 and P but not with D15	0
Relief with D2 and D15 but not with P	1
Relief with P and D15 but not with D2	0
Relief with D2, P, and D15	2
Total	8

* D2 and P as noted in Table 1.
D15 refers to drug administered at the 15-mg. level every hour for four hours.

superiority of the drug, regardless of dosage, over the placebo. Furthermore, the findings are of such a nature that there would seem to be little likelihood that increasing the number of cases would alter the outcome.

The results of this study fail to indicate that oral Releasin, in dosages as high as 15 mg. every hour for four hours, has any significant influence upon the discomfort associated with primary dysmenorrhea.

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REFERENCES

1. ABRAMSON, D., and REID, D. E.: Use of relaxin in treatment of threatened premature labor, *J. Clin. Endocrinol. & Metab.* **15**: 206, 1955.
2. ABRAMSON, D., and REID, D. E.: Primary dysmenorrhea; treatment with relaxin, *Obst. & Gynec.* **12**: 123, 1958.

RESIN UPTAKE OF RADIOTHYROXINE IN SERA FROM NON-PREGNANT AND PREGNANT WOMEN

TO THE EDITOR:

While using radiothyroxine in the course of dialysis equilibrium studies it was noted that significant but varying amounts of the labeled thyroxine adhered to the dialysis membrane. This suggested that a system might be designed in which a synthetic material could compete against sera for radiothyroxine. Such a model would serve to measure quantitative differences in the binding behavior of specific serum proteins. Previous studies have demonstrated that certain resins have marked affinities for inorganic iodide, non-thyroxine iodine, and thyroxine (1–3). Therefore, Amberlite IRA-400, a strongly basic anion exchange resin[1] was selected to compete against the sera from non-pregnant and pregnant women for radiothyroxine. It has been shown by paper electrophoresis at pH 8.6 that the alpha-globulin fraction of sera from pregnant women has an enhanced ability to bind thyroxine, and that significantly less of the circulating thyroxine is in the "free" state (4,5).

Sera were obtained from 69 pregnant women whose periods of uncomplicated gestation varied from 7 to 36 weeks. Control sera were collected from 74 non-pregnant women in the childbearing age. The resin, which had been cycled in the acetate or chloride form (pH 5.5–6.0), was stored wet in a glass container until used. One-ml. volumes of the damp resin were measured into graduated Pyrex test tubes (16 mm. × 100 mm.) by means of a 10-ml. syringe with the end removed to facilitate uniform delivery. The resins were washed three times with a jet of distilled water, allowing time for the particles to resettle before the rinse was removed by suction.

One-ml. aliquots of sera (from non-pregnant and pregnant women) containing 0.02 to 0.03 microcurie of pure[2] *l*-thyroxine-I^{131} and less than 0.01 microgram of additional stable *l*-thyroxine were added alternately in duplicate to the resin tubes. Initially the tubes were shaken at room tempera-

[1] Rohm and Haas Co., Philadelphia 5, Pa.

[2] Chromatographically pure, obtained from Abbott Laboratories, Oak Ridge, Tenn.

ture, but because of the temperature dependence of the system, subsequent equilibrations were carried out at 3° C. The tubes, inclined at a 30-degree angle, were shaken for one and a half hours at 60 cycles per minute and then removed for counting in a conventional well-type scintillation detector. Following this, the resins were washed with a stream of distilled water sufficient to remove the serum-bound radioactivity. The radioactivity remaining on the resin divided by the radioactivity of the unwashed resin measured the fraction of labeled thyroxine adsorbed. The mean difference between duplicate determinations was 2.2 ± 1.6 per cent, and the duplicate variability for 95 per cent of the determinations was less than 5.4 per cent.

It became apparent during the course of these experiments that there was considerable fluctuation in the numerical values for identical groups of sera. Although the reasons for the inconstant values are not altogether clear, subsequent investigation revealed that the rate of equilibration between resin and serum thyroxine-I^{131} was more rapid at higher temperatures. Other factors, such as the equilibration time, cycled form of the resin, changes in resin pH, and the degree of resin impurity with regard to deterioration, appeared to influence the values. In an attempt to attain more uniform results, experimental conditions were altered and later experiments were carried out at 3° C. instead of at room temperature, using both the acetate and chloride resin cycle. Thus, comparisons have been made between determinations in control and pregnant groups which were performed at the same time and under similar conditions. Sera from the pregnant and non-pregnant women, which were examined together, were grouped into 6 divisions on the basis of the experimental conditions, *viz*, equilibrium temperatures, resin pH, and cycled form of the resin. Figure 1 depicts the mean values for the individual sera in each group. Examination of the divisions reveals that in each instance the percentage of radiothyroxine taken up by the resin was significantly lower in the pregnancy groups than in the non-pregnant controls. Overlap of values in the 2 groups was not observed, although 1 control determination (Division IV) was in the pregnancy range.

In the foregoing experiments, thyroxine had been added in physiologic concentrations. A separate experiment was carried out in which both groups of sera were enriched by adding graded amounts of stable thyroxine up to 500 μg. per 100 ml. of serum. The resin uptake of radiothyroxine was substantially increased in both groups, but at all except extreme concentrations of added thyroxine the values for the pregnancy sera were significantly lower.

The results suggest that in pregnancy serum it is either the increased affinity of thyroxine-binding protein for the hormone or else a quantitative

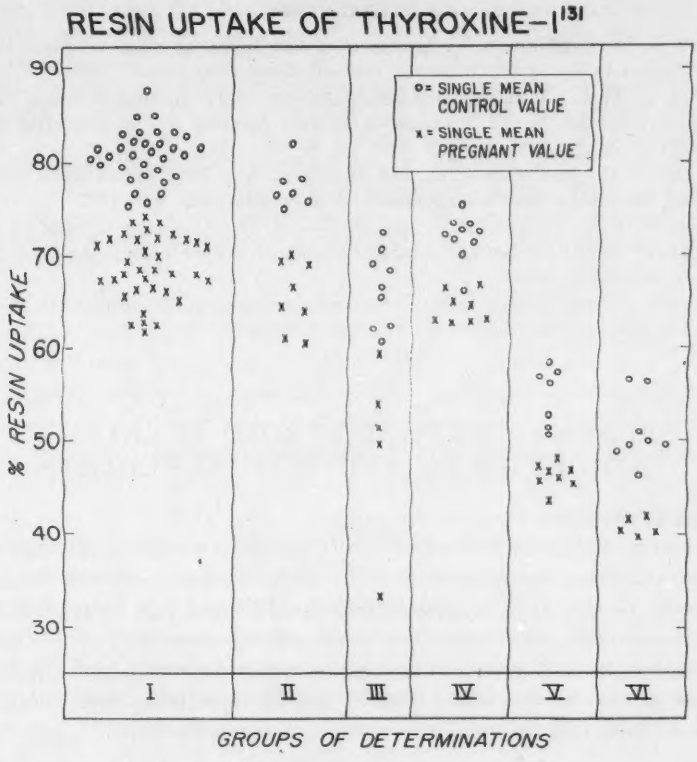

FIG. 1. Resin adsorption of labeled thyroxine from sera.
Divisions I and II—Equilibration at room temperature; resin in the acetate form.
Divisions III and IV—Equilibration at 3° C.; resin in the acetate form.
Divisions V and VI—Equilibration at 3° C.; resin in the chloride form.

protein increment that reduces the amount of thyroxine available to the resin. Whether other thyroid hormones and other kinds of sera will interact with the resin in specific fashion is currently under study.

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REFERENCES

1. Scott, K. G., and Reilly, W. A.: Use of anionic exchange resins for the determination of protein-bound I^{131} in human plasma, *Metabolism* **3**: 506, 1954.
2. Mitchell, M. L.; Burrows, B. A., and Graham, D. E.: Binding of iodide by serum albumin. Abstract No. 61, Program of the 39th Meeting of The Endocrine Society, New York, N. Y., 1957.
3. Ingbar, S. H.; Dowling, J. T., and Freinkel, N.: The purification of thyroxine-binding protein by ion-exchange resins, *Endocrinology* **61**: 321, 1957.
4. Dowling, J. T.; Freinkel, N., and Ingbar, S. H.: Thyroxine-binding by sera of pregnant women, new-born infants, and women with spontaneous abortion, *J. Clin. Invest.* **35**: 1263, 1956.
5. Robbins, J., and Nelson, J. H.: Thyroxine-binding by serum proteins in pregnancy and the new-born, *J. Clin. Invest.* **37**: 153, 1958.

PLASMA CORTICOSTEROID LEVELS IN AIRCREWMEN AFTER LONG FLIGHTS

To the Editor:

Prolonged flights in military aircraft result in a state of fatigue which, subjectively, may be extreme; yet the underlying physiologic changes are unknown. Evidence of adrenocortical involvement has been obtained by Marchbanks (1), who noted increased urinary excretion of 17-hydroxycorticosteroids in a group of aircrewmen during a 22½-hour flight. The purpose of the present study was to test the possibility that blood corticosteroid levels in fatigued aircrewmen become elevated.

METHODS

The fluorescence method of Sweat (2) for the detection of hydrocortisone and a corticosterone-like fraction was employed. Pre- and post-flight venous blood samples were obtained from 44 men (members of 9 crews) participating in training flights in B-52 aircraft lasting nine to twelve hours. All flights began in the morning—the earliest one starting at 0730 and the latest at 1200. Pre-flight blood samples were taken at least one and a half hours before the start of the flight, because this was the time at which the crews entered their aircraft to begin preflight preparations and they were not again approachable before takeoff. Post-flight blood samples were taken about thirty minutes after the end of the flights. All of the flights were rated as successful and without any unusual event. Cabin altitude at no time exceeded 10,000 feet.

RESULTS

Mean pre-flight values for each of the 2 steroid fractions (Table 1) were in excellent agreement with those obtained by Sweat (2) for 21 normal male subjects. His values were 10.8 ± 0.6 and 4.3 ± 0.5 μg. per 100 ml. of plasma for hydrocortisone and the corticosterone-like fraction, respec-

TABLE 1. PLASMA CORTICOSTEROID LEVELS IN RELATION TO FLYING FATIGUE

Corticosteroid*	Pre-flight	Post-flight	Gain
Hydrocortisone	11.3±0.45	16.8±0.75	+5.4±0.57 (P<.01)
Corticosterone-like fraction	4.8±0.22	7.1±0.36	+2.3±0.30 (P<.01)

* Values are means with standard error (n=44) and are expressed as μg./100 ml. of plasma.

tively. Mean post-flight values for each of the fractions proved to be significantly different from Sweat's values ($P < .01$). The mean gain for each of the 2 fractions was analyzed statistically and found to differ significantly from zero ($P < .01$). In only 1 of the 44 subjects was there a decrease, and this involved hydrocortisone only. In this particular case, pre-flight values for both fractions were relatively high (20.8 and 7.4 μg. for hydrocortisone and the corticosterone-like fraction, respectively). Although the hydrocortisone value after the flight (19.2 μg.) was lower than previously, it was still a relatively high value. The final corticosterone-like fraction after the flight (8.1 μg.) was also relatively high.

Mean values for those crewmembers who were instructors did not differ significantly from those who were in training; nor were there significant differences between crewmembers in different categories, namely, Aircraft Commander, Pilot, Navigator, Electronics Countermeasure Operator, and Gunner.

DISCUSSION

In attempts to assess flying fatigue during long-range flights, Murphy, Gofton and Cleghorn (3) used an older method for the determination of urinary corticoids and obtained equivocal results. With a more sensitive and precise method, Marchbanks (1) found the urinary 17-hydroxycorticosteroid values during an extremely long flying mission (twenty-two and a half hours) in a B-52 aircraft to be significantly higher than on non-flying days. In the present study on B-52 crewmembers, flight times were approximately half as long as in Marchbanks's study; still, both sets of results suggest functional changes involving corticosteroids. Although it would have been advantageous to have obtained urinary samples along with the blood samples in the present study, operational problems prevented us from doing so. Furthermore, although blood determinations on non-flying days would have been of great value, scheduling difficulties made this impractical. Nevertheless, the use of pre-flight values estab-

lished that, as a group, these men had normal blood levels of corticosteroids initially and that a significant increase occurred. That this increase was not of the nature of a diurnal variation is clear, since others (4, 5) have found that plasma 17-hydroxycorticosteroid levels in normal persons tend to decrease throughout that period of the day in which these flights took place. Since Romanoff and associates (6) noted that the diurnal rhythm in urinary excretion of total 17,21-dihydroxy-20-ketosteroids, tetrahydrocortisol, and tetrahydrocortisone in Air Force bomber crewmembers engaged in flying activities did not differ from that of non-flying, normal subjects, it appears that urinary and blood indices of adrenocortical function are not equally sensitive.

Sweat (2) stated that the ratio for the 2 fractions of steroids varies widely among individuals and does not appear to conform to any pattern. However, the mean pre-flight hydrocortisone/corticosterone-like ratio for the subjects in the present series was 2.40 ± 0.068 and the post-flight value was 2.42 ± 0.080. For individuals, pre- and post-flight ratio values varied and different patterns of change were seen, but for the group the constancy was striking—especially so because the method employed for the corticosterone-like fraction yields values which are high as compared with those obtained by Peterson's method (7) for corticosterone.

From the report of Brown, Englert and Wallach (8), that plasma concentrations of free 17-hydroxycorticosteroids are influenced more by the rate of removal from plasma than by the adrenal response to corticotropin (ACTH) (at least in patients with metabolic disorders) , it does not seem safe to interpret these elevations in plasma corticosteroid levels in fatigued men as evidence of adrenocortical stimulation. Wallace, Christy and Jailer (9) observed patients with renal disease who had elevated blood levels of corticosteroids and low urinary values; thus under certain conditions the 2 measures may fail to agree in their indications. We do not imply that renal or metabolic disorders develop because of flying activities, but the foregoing points are mentioned to emphasize that the changes in plasma steroid fractions may represent changes other than those resulting from the release of ACTH by the pituitary.

SUMMARY

The fluorescence method of Sweat for the determination of hydrocortisone and a corticosterone-like fraction in blood was utilized in an attempt to evaluate flying fatigue in a group of 44 aircrewmen participating in flying activities in military aircraft. Mean pre-flight values for each of the two steroid fractions agreed with those reported by Sweat for normal male subjects, but significant increases in both fractions were noted after flights

of nine to twelve hours' duration. This change was not of the nature of a diurnal variation.

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August 9, 1958

REFERENCES

1. MARCHBANKS, V. H., JR.: Urinary 17-hydroxycorticosteroid levels in B-52 crews in relation to stress on a 22½ hour mission, *J. Aviation Med.* (in press).
2. SWEAT, M. L.: Adrenocorticosteroids in peripheral and adrenal venous blood of man, *J. Clin. Endocrinol. & Metab.* **15**: 1043, 1955.
3. MURPHY, C. W.; GOFTON, J. P., and CLEGHORN, R. A.: Effect of long-range flights on eosinophil level and corticoid excretion, *J. Aviation Med.* **25**: 242, 1954.
4. MIGEON, C. J.; TYLER, F. H.; MAHONEY, J. P.; FLORENTIN, A. A.; CASTLE, H.; BLISS, E. L., and SAMUELS, L. T.: The diurnal variation of plasma levels and urinary excretion of 17-hydroxycorticosteroids in normal subjects, night workers and blind subjects, *J. Clin. Endocrinol. & Metab.* **16**: 622, 1956.
5. BROWN, H.; ENGLERT, E., JR.; WALLACH, S., and SIMONS, E. L.: Metabolism of free and conjugated 17-hydroxycorticosteroids in normal subjects, *J. Clin. Endocrinol. & Metab.* **17**: 1191, 1957.
6. ROMANOFF, L. P.; RODRIGUEZ, R. M.; SEELYE, J. M., and PINCUS, G.: Determination of tetrahydrocortisol and tetrahydrocortisone in the urine of normal and schizophrenic men, *J. Clin. Endocrinol. & Metab.* **17**: 777, 1957.
7. PETERSON, R. E.: Plasma corticosterone and hydrocortisone levels in man, *J. Clin. Endocrinol. & Metab.* **17**: 1150, 1957.
8. BROWN, H.; ENGLERT, E., JR., and WALLACH, S.: Metabolism of free and conjugated 17-hydroxycorticosteroids in subjects with thyroid disease, *J. Clin. Endocrinol. & & Metab.* **18**: 167, 1958.
9. WALLACE, E. Z.; CHRISTY, N. P., and JAILER, J. W.: Clinical application of the simplified Silber-Porter method for determining plasma 17-hydroxycorticosteroids, *J. Clin. Endocrinol. & Metab.* **15**: 1073, 1955.

The Endocrine Society

THE 1959 ANNUAL MEETING

The Forty-First Annual Meeting of The Endocrine Society will be held in the Haddon Hall Hotel, Atlantic City, New Jersey, Thursday, Friday, and Saturday, *June 4, 5 and 6, 1959.*

The Chairman of Local Arrangements is Dr. Matthew Molitch.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily, and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 5, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Chalfonte-Haddon Hall Hotel will hold 300 bedrooms for members until May 1, 1959, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations now directly with the hotel, advising them of time and date of arrival and departure. Avoid disappointment.

Final program, membership card and advance registration forms will be sent on May 1, 1959 to members whose current dues have been paid.

Those wishing to present papers, which will be strictly limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Frank Engel, Duke Hospital, Durham, North Carolina, not later than *February 1, 1959. It is imperative that the abstracts be informative and complete with results and conclusions*—not a statement that those will be presented at the meeting—in order that they may be of reference value and suitable for printing in the program. The reading and processing of approximately two hundred abstracts submitted each year and the compiling of the program from these involves a tremendous amount of time and effort on the part of the Program Committee. The Council requests that the authors adhere strictly to the following rules when submitting abstracts, otherwise they cannot be considered:

1. *It is assumed that abstracts submitted for this program have not and will not be submitted elsewhere.*
2. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the

text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.

3. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of the nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be written after his name.
   Line 3. Institution of origin and city in which institution is located.
4. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
5. Abstracts should be letter perfect, since there will be no opportunity for proof reading by the authors.

THE 1959 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

FRED CONRAD KOCH AWARD

During the past year a substantial legacy has been bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is to be the highest honor of The Endocrine Society and is to be represented by a medal that is to be known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award will replace the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society.

Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

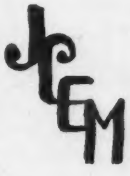
The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.



The American Goiter Association

THE 1959 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Drake Hotel, Chicago, Illinois, on *April 30, May 1 and 2, 1959.*

THE VAN METER PRIZE AWARD FOR 1959

The American Goiter Association again offers the Van Meter Prize Award of $300.00 and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The award will be made at the annual meeting of the Association which will be held in the Drake Hotel, Chicago, Illinois, April 30, May 1 and 2, 1959.

The competing essays may cover either clinical or research investigations, should not exceed 3,000 words in length and must be presented in English. Duplicate typewritten copies, double spaced, should be sent to the Secretary, Dr. John C. McClintock, 149½ Washington Avenue, Albany 10, New York, not later than *January 15, 1959.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the winning essay by the author if it is possible for him to attend.

JOHN C. MCCLINTOCK, M.D.
Secretary

BOOK REGISTER

Adénomes hypophysaires. By GÉRARD GUIOT, Neurosurgeon of the Foch Foundation, and G. ARFEL, S. BRION, J. CHEGUILLAUME, A. ENNUYER, J. FANJOUX, H. FISCHGOLD, L. GUILLAUMAT, J. METZGER, J.-P. MICHARD, B. THIBAUT, P. TOURNOUX and G. FOURC'H. Symposium presented at the Annual Reunion of the Society of Neurosurgery, French Language, 1958. The plan of presentation is as follows: General study, endocrinology, ophthalmology, clinical aspects, radiology, electroencephalography, histology, surgical treatment, anesthesiology, radiotherapy, use of radioactive isotopes, and bibliography; 276 pages; 118 figures; 6 plates; 1958. Masson et Cie, Éditeurs, Paris, France. Price, 3,800 fr.

Annotated Bibliography of Vitamin E. Volume IV, 1955–1957. Compiled by D. C. HERTING, W. F. KUJAWSKI, M. L. LUDWIG and P. L. HARRIS of the Research Laboratories of Distillation Products Industries, Division of Eastman Kodak Company, Rochester, N.Y. Contains 1384 listings and abstracts on the subject of Vitamin E.; 216 pages; 1958. Distributed by The National Vitamin Foundation, Inc., 149 East 78th St., New York 21, N.Y. Price $3.00.

(*The*) *Chemical Prevention of Cardiac Necrosis.* By HANS SELYE, M.D., Ph.D., D.Sc., Professor and Director of the Institute of Experimental Medicine and Surgery, University of Montreal, Montreal, Canada. This book should be of interest to endocrinologists, cardiologists, and physiologists. It provides a summary of the current clinical and experimental observations on cardiac necroses, viewed in the light of new knowledge about the relationships between electrolytes, steroids and stress in animals. Cardiac necroses can be produced in animals by a variety of drugs, physical agents, allergens and infections, and the necrotizing myocarditis is uniformly influenced by corticoids and electrolytes. On this basis, the author believes it is probable that some of the spontaneous necrotizing cardiopathies in man may also be beneficially influenced by one of the more common electrolytes, and that this type of treatment may be applicable in the prevention of acute cardiac death following stress. The aspects of the subject covered in the various chapters are: Historical introduction, pathogens and conditioning factors of cardiac necroses, electrolytes, hormones, vitamins, cardiac glycosides and their aglycones, other chemical compounds and food constituents, physical agents including surgical intervention, psychic and nervous stimuli, microbes and their toxins, hypersensitivity, spontaneous diseases, various other factors, theories, outlook, summary, conclusions, and an extensive bibliography of the world literature on cardiac necroses; 250 pages; 24 illustrations including a color plate; 15 tables; 1958. The Ronald Press Company, 15 East 26th Street, New York 10, N.Y. Price $7.50.

Clinical Obstetrics and Gynecology. Vol. I, No. 3, (Sept. 1958). This volume of a quarterly series contains a *Symposium on Special Diagnostic Aids* edited by C. PAUL HODGKINSON, M.D., Gynecologist-Obstetrician-in-Chief, Henry Ford Hospital, Detroit, Michigan, with several contributors; and a *Symposium on Abnormal Uterine Bleeding* edited by JOHN I. BREWER, M.D., Professor of Obstetrics and Gynecology, Northwestern University Medical School, Chicago,

Illinois, with several contributors; 852 pages; illustrated; 1958. Paul B. Hoeber, Inc., 49 East 33rd Street, New York 16, N.Y. By subscription, $18.00 for four consecutive numbers.

Metabolic Disturbances in Clinical Medicine. Edited by G. A. SMART, B.Sc., M.D., F.R.C.P., Professor of Medicine, University of Durham, Newcastle upon Tyne, with the collaboration of 14 experts. This book concerns the diagnosis and treatment of the more important metabolic diseases, and attempts a balance between the purely clinical and the purely biochemical approach, within the bounds of a volume that is of such size as to be readily useful. Chapters are included on: Problems of general nutrition in clinical medicine, disturbances of water and electrolytes, metabolic aspects of renal disease, metabolic disturbances following injury, metabolic aspects of liver disease, cardiovascular aspects of metabolic disease, edema in chronic congestive heart failure, atherosclerosis, lung function and acid-base balance, oxygen therapy, metabolic bone disease, metabolic disturbances and the hematopoietic system, and disturbances of metabolism affecting the central nervous system; 358 pages; illustrated; 1958. Little, Brown and Company, Boston, Massachusetts. Price $10.00.

Métabolisme de l'eau dan les traumatismes du crâne. Aspects neuroendocriniens et base du traitement hormonal. By D. PETIT-DUTAILLIS, Professor of Neuro-surgery, and E. BERNARD-WEIL, Chief, Neurosurgical Clinic, both of the Faculty of Medicine of Paris. Symposium presented at the Annual Reunion of the Society of Neurosurgery, French Language, 1958. The 6 chapters cover: The metabolism of water, regulation of water-electrolyte metabolism from the point of view of endocrinology, summary of work on disorders of water metabolism (surgical and neuro-surgical), methods of examination, case reports, and treatment. There is also a bibliography of 154 items; 108 pages; 10 figures; 1958. Masson et Cie, Éditeurs, Paris, France. Price 1,400 fr.

Metabolism of Lipids. A Symposium in *British Medical Bulletin* Volume 14, Number 3, September 1958, by 20 leading British investigators. Professor A. C. Frazer, who has written the introduction, remarks, "The papers presented here do not cover all aspects of lipid metabolism, but they touch on most of the major issues that are in the forefront of advancing knowledge at the present time. The volume is concise and filled with new facts and formulations. The various papers cover the following subjects: Lipid metabolism, biosynthesis of fatty acids, hormonal effects in vitro on lipogenesis in mammary tissue, fat absorption and its disorders, biosynthesis of cholesterol, the absorption and metabolism of sterols, removal of lipids from the blood stream, hormonal control of the circulating lipids, the effect of dietary fats on the blood lipids and their relation to ischemic heart disease, fats and blood coagulation, essential fatty acids and the skin, essential fatty acids and human disease, and lipids and the structure of myelin. Published by the Medical Department. The British Council, 65 Davies Street, London, W.1, England. Price $3.25.

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DECADRON "possesses greater anti-inflammatory potency per milligram than any steroid yet produced,"[1] and is "the most potent steroid thus far synthesized."[2] Milligram for milligram, it is, on the average, 5 times more potent than 6-methylprednisolone or triamcinolone; 7 times more potent than prednisone; 28 times more potent than hydrocortisone; and 35 times more potent than cortisone.

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Virtual **absence of diabetogenic activity, edema, sodium or water retention, hypertension, or psychic reactions** has been noted with DECADRON.[1,2,3,4] Other "classical" reactions were less frequent and less severe. DECADRON showed no increase in ulcerogenic potential, and **digestive complaints were rare. Nor have there been any new or "peculiar" side effects,** such as muscle wasting, leg cramps, weakness, depression, anorexia, weight loss, headache, dizziness, tachycardia, or erythema. Thus DECADRON introduces **a new order of magnitude in safety,** unprecedented in corticosteroid therapy.

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References:
1. Boland, E. W.: California Med. 88:417 (June) 1958. 2. Bunim, J. J., et al.: Arthr. & Rheum. 1:313 (Aug.) 1958 3. Boland, E. W., and Headley, N. E.: Paper read before the Am. Rheum. Assoc., June 21, 1958, San Francisco, Calif. 4. Bunim, J. J., et al.: Paper read before the Am. Rheum. Assoc., June 21, 1958, San Francisco, Calif.

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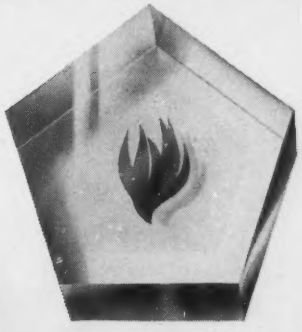

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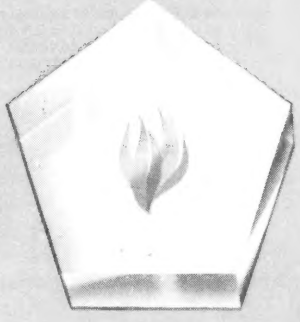

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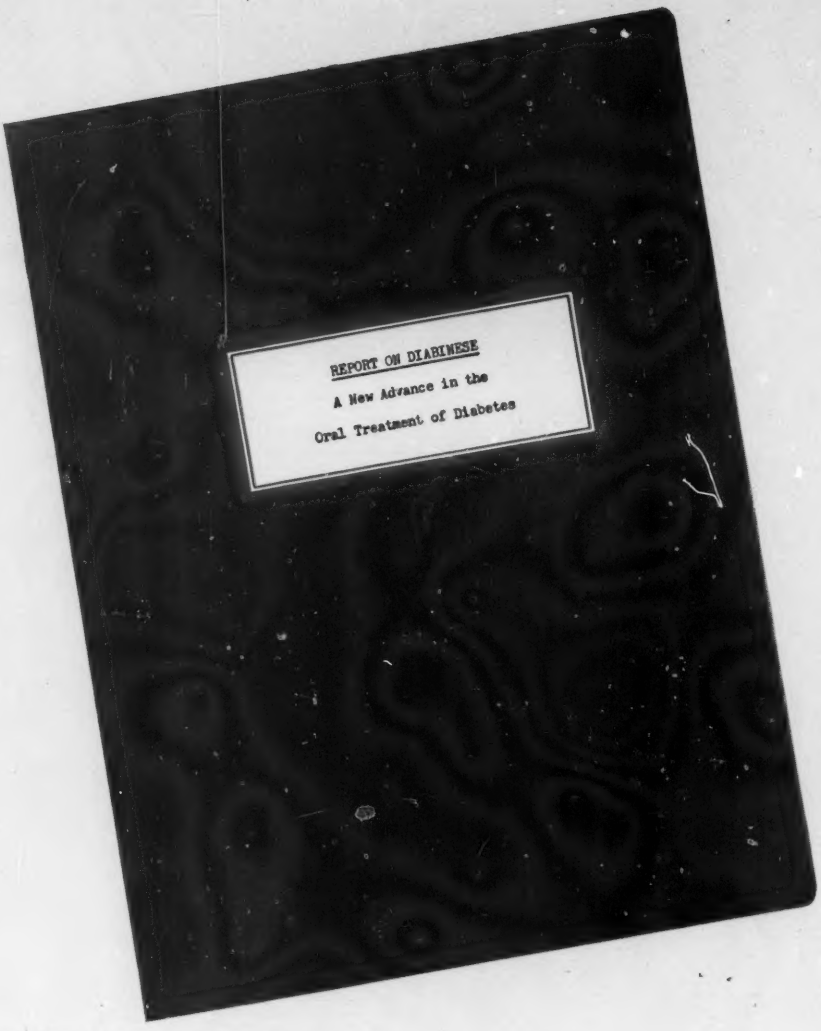

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You can't make her 35 again—but the odds are good that you can make her **feel** like it! The secret is a combination of reassurance and hormones. The exact form and amount of the former defy objective analysis, but the latter can now be provided with scientific precision. Reduced to essentials, here is the explanation of exactly how hormones—in the form of Upjohn's new Halodrin—restore the "premenopausal prime."

The normal premenopausal woman **excretes** estrogens in the urine in the form of estradiol, estrone, and estriol, in an approximate 28-day average ratio of 39:15:46. Starting with this urinary excretion of estrogens, it is possible to calculate backwards and estimate the amount of estradiol that must have been secreted endogenously in order to produce these urinary levels. This is possible because the proportion of estrogens which appears in the urine following parenteral administration has been established in castrated women.

On this basis, the average endogenous output of estrogens is about 160 micrograms per day during a menstrual cycle, and 80 micrograms per day in postmenopausal women (see chart opposite). Therefore, the restoration of the "premenopausal prime" in the postmenopausal woman requires the replacement of approximately the equivalent of the 80 micrograms of estradiol per day that she no longer secretes endogenously.

Oral ethinyl estradiol is about 2 to 2½ times as potent as **parenteral** estradiol. Therefore, **the replacement of 80 micrograms of endogenous estradiol production per day is accomplished by the oral administration of 32 to 40 micrograms of ethinyl estradiol per day.**

Each Halodrin tablet contains 20 micrograms of ethinyl estradiol, which means that the recommended dosage of 2 tablets per day provides 40 micrograms of ethinyl estradiol. This offsets the loss of 80 micrograms of endogenous estradiol production in the menopausal woman; i.e., restores the "premenopausal prime."

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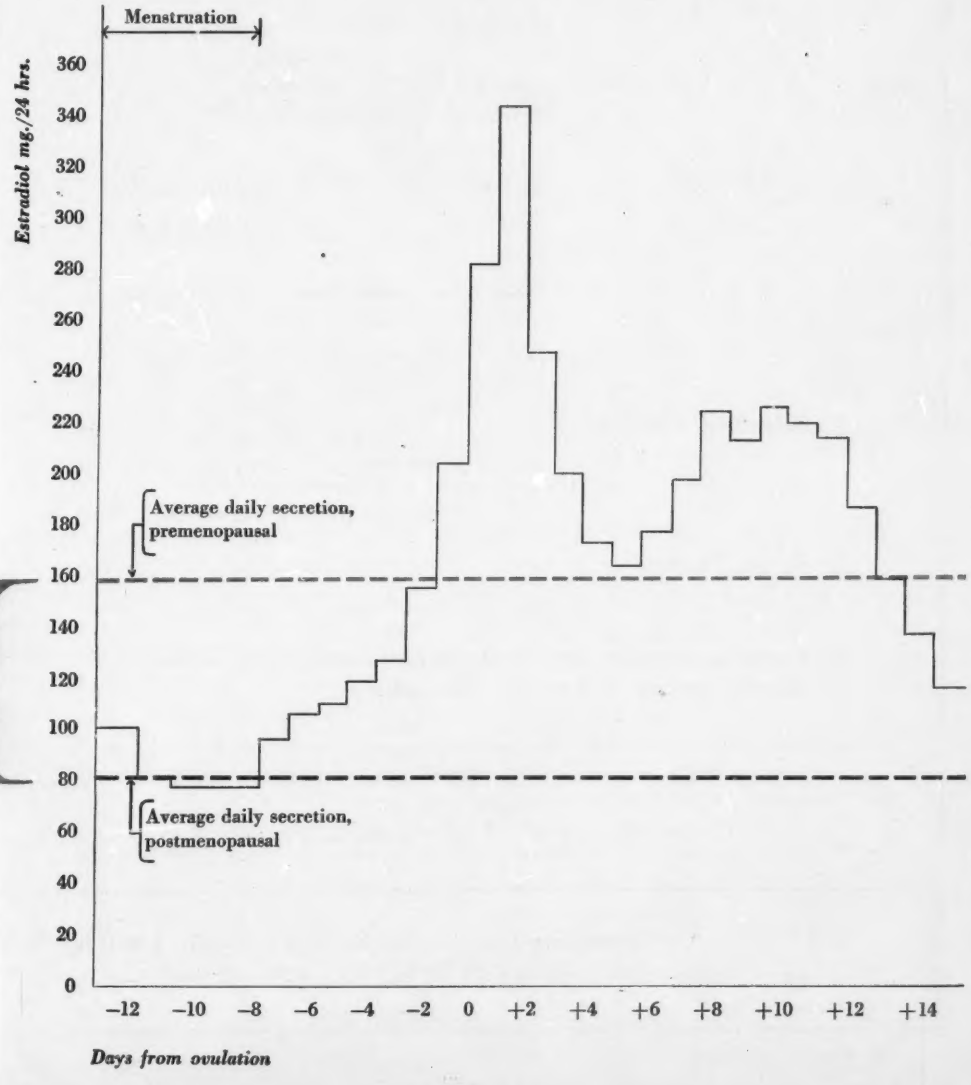
Endogenous estrogen secretion (mg./24 hours)
(calculated from average 24-hour urinary excretion of estradiol, estrone, and estriol)



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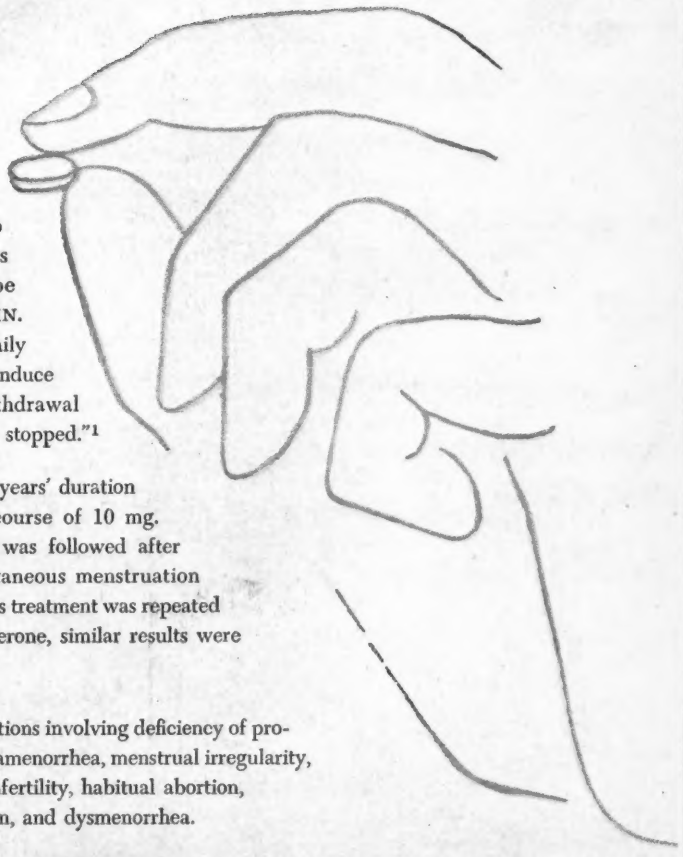

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REFERENCES:
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(2) Hertz, R.; Waite, J. H., & Thomas, L. B.: *Proc. Soc. Exper. Biol. & Med.* 91:418, 1956.

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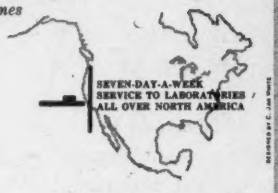

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